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THE UNIVERSITY OF CHICAGO PRESS
CHICAGO, ILLINOIS, U.S.A.

PHYSIOLOGICAL ZOÖLOGY

A Quarterly Journal of Zoölogical Research

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Business correspondence should be addressed to The University of Chicago Press, Chicago 37, Ill.

Communications for the editors and manuscripts should be addressed to W. C. Allee, Managing Editor of PHYSIOLOGICAL ZOÖLOGY, The University of Chicago, Chicago 37, Ill.

The articles in this journal are indexed in the *International Index to Periodicals*, New York, N.Y., the *Quarterly Cumulative Index Medicus*, and *Biological Abstracts*.

Applications for permission to quote from this journal should be addressed to The University of Chicago Press, and will be freely granted.

Entered as second-class matter October 27, 1928, at the post-office at Chicago, Illinois, under the Act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in United States Postal Act of October 3, 1917, Section 1103, amended February 28, 1925.

[PRINTED IN U.S.A.]

Charles Vincent Taylor

1885-1946

Charter Member of the Editorial Board
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PHYSIOLOGICAL ZOÖLOGY

Volume XIX APRIL 1946 Number 2

ORGANIZERS IN DEVELOPMENT AND THE ORGANIZER CONCEPT

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AN ORGANIZER is something that organizes something else. The organizer concept, as generally accepted in developmental physiology, has been constructed primarily on the foundation of experimental analysis of amphibian development. Some authors apparently regard results of experiment on amphibian development as an adequate foundation for a general theory of development, and the amphibian organizer as, in a sense, a model for organizers in general. Such a viewpoint seems not only narrow but misleading. Experimental evidence has shown that even the so-called "primary" organizer of amphibians is not primary, but merely a step in the progress of organization of the embryo. It does not initiate organization but is itself a product of preceding organization. If we are to attain an adequate theory of development and to throw any real light on the problem of organization, we must endeavor to discover the real initiators of organization and base further analysis on what we find. If an organizer is something that organizes,, organizers or reorganizers are not only parts of embryos but may be conditions to which the egg cell or oöcyte or other reproductive cell or multicellular mass is exposed in the body of the parental organism. In reconstitution, organizers, or, strictly speaking, reorganizers, may be blastemas or primordia or certain parts. These are reorganizers because they make over the pre-existing organization. And, finally, differentials in extraorganismic factors may be organizers or reorganizers. This paper is a survey and discussion of some of the experimental data bearing on the organizer problem and the conclusions to which they point.[1]

ORGANIZERS OF THE *Fucus* EGG AND OF CERTAIN OTHER PLANTS

The egg of the alga *Fucus* is spherical before development begins. The first evidence of development is a slight deformation appearing in its earliest stages as a slight protuberance of one region without visible structural difference from other parts (Fig. 1, *A*). This elongation

[1] The writer is indebted to the University of Chicago Press for permission to use the following figures reproduced from earlier special papers in his book *Patterns and Problems of Development* (1941): Figs. 57, *A–K;* 119, *B–G;* 121, *B–D;* 122, *A–F, H, I;* 125, *D;* 130, *B;* 131, *A, B;* 141, *A, D;* 142, *A–H;* 144; 212, *B–D;* 215. Acknowledgment of sources is also made in the legend of each figure. These figures are reproduced again here because they are regarded as highly significant in relation to problems of organization and polarity.

in one diameter becomes a definite outgrowth (Fig. 1, *B*); and division into two cells in a plane at right angle to the axis of elongation follows (Fig. 1, *C*), the cell constituting the elongating region becoming the primary rhizoid, the organ of attachment, the other cell giving rise to the thallus. The rhizoid undergoes further elongation, and both rhizoid and thallus cells divide further (Fig. 1, *D* and *E*). The recently fertilized eggs stick to a solid surface; they may also be imbedded in agar to prevent possible change in position, and so can easily be subjected to various external differentials. It has been known for almost fifty years that their polarity, as indicated by the axis of rhizoid outgrowth and the plane of the first cell division, can be determined by differential illumination, the rhizoid developing on the less illuminated side. This differential is an organizer. Later it was found that a range of wave-lengths toward the blue-violet was most effective as organizer. Electric current was also found to be an organizer of this egg, the rhizoid developing toward the anode. Still more recently it has been shown, chiefly by the experiments of Whitaker and his co-worker, Lowrance, that other differentials can act as organizers of this egg. The eggs of at least some species will develop in complete darkness without evident relation to any definite external differential, though less rapidly than when subjected to some definite effective differential. This suggests that the free eggs possess some polarity independently of external factors. If so, it is probably determined in the parent thallus. If this is the case, the external organizers are really reorganizers. Some of the differentials found to be effective are noted. It had been observed earlier, that when several eggs lie close together, their polarities are determined with reference to each other, rhizoids appearing toward the center of the group. This group effect is increased by increase of H-ion concentration, and a gradient of H-ion concentration induces rhizoid development on the more acid side up to a certain con-

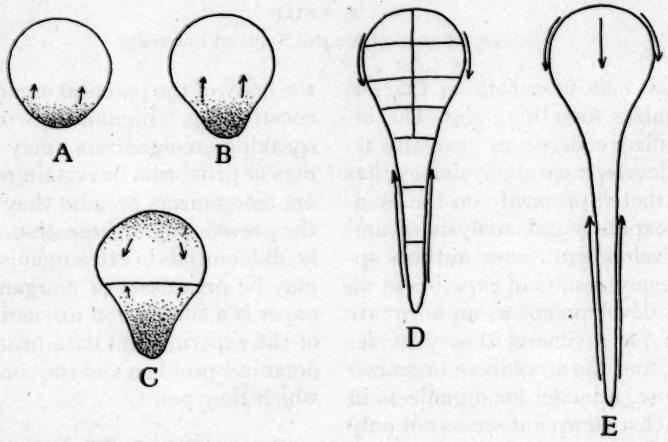

FIG. 1.—Indophenol gradients in developmental stages of the alga *Fucus*. *A*, early, *B*, later, stage of rhizoid development with gradient from rhizoid tip; *C:* two-cell stage, gradient from rhizoid tip and slight gradient from apical region of thallus cell; *D*, *E*, later, multicellular stages, gradients indicated by arrows.

centration and, above that, on the less acid side. The rhizoid tends to form on that side of the egg to which certain concentrations of the potassium salt of indoleacetic acid (heteroauxin) are applied. These experiments suggested that at least some external differentials might organize or reorganize by their differential effect on auxin activity in the egg. For example, increase of pH increases auxin activity, and very high H-ion concentrations may perhaps increase it so much that it becomes inhibitory. After certain intensities and periods of centrifuging, eggs with any possible orientation prevented, and in which stratification persists, develop rhizoids at or near the centrifugal pole at the H-ion concentration of sea water; but in somewhat acid sea water most rhizoids develop at the centripetal pole. Eggs exposed to a slight temperature gradient develop rhizoids on the warmer side. Recently fertilized eggs, drawn into a capillary pipette with lumen small enough to produce elongation, retain the elongated form on extrusion after "setting" of the cellulose and tend to develop rhizoids at or near one end of the longitudinal axis in sea water, but at pH 6 rhizoids tend to form on the surface in contact.[2] As soon as the eggs show the slightest deformation indicating rhizoid development, and apparently even before deformation is evident, the indophenol reaction shows a very distinct gradient with high end in the rhizoid region (Fig. 1, *A*); and as the rhizoid elongates, the reaction gradient, decreasing from its tip, appears before reaction is evident in the thallus cell (Fig. 1, *B*). In some individuals a slight reaction gradient, decreasing from the apical region, appears in the thallus cell at the stage of Figure 1, *C*. In later stages the rhizoid gradient persists and the thallus gradient becomes progressively more distinct (Fig. 1, *D* and *E*). Reduction in low oxygen in darkness shows the same gradient pattern as the reaction unless intracellular concentration of indophenol has become high enough to injure more rapidly reacting regions and so retard or prevent reduction in them. On exposure to light more or less reoxidation occurs, presumably in consequence of photosynthesis. The rhizoid oxidation-reduction gradient pattern is the same as the differential susceptibility pattern, but in the thallus the oxidation-reduction pattern appears somewhat earlier than the susceptibility pattern. Evidently, rhizoid development is an expression of a polar organization and the oxidation-reduction pattern is the earliest direct evidence of that organization thus far discovered. Since most of the external organizing or reorganizing factors are quantitative differentials, it appears highly probable that the organization determined is primarily a quantitative gradient pattern and that the indophenol gradient pattern is a visible expression of certain characteristics of it.

With frequently repeated change of position with respect to direction of incident sunlight the rhizoid axis and the division plane may form any angle with each other, and bipolar forms frequently result from changes in position of 180° (Child, 1941*a*, pp. 423, 424). Apparently, determination of rhizoid position and of the plane of the later cell division require different intensities or periods of differential illumination, and different partial axes can evidently be determined in the same individual.

The point is to be emphasized that it is, not the extraorganismic factors (light, pH, etc.) as such, but their pattern or differential of action, that organizes. It

[2] For further data and references see Child, 1941*a*, pp. 423–25; also Needham, 1942, pp. 668, 669.

appears highly probable that they organize a gradient pattern which is primarily quantitative, and the differential growth and the oxidation-reduction pattern indicate that quantitative metabolic differentials are an essential feature of the pattern.

In the regeneration of the axiate alga, *Bryopsis*, polarity can be determined by light; and in the spore of *Equisetum*, organization and plane of the first division are determined by differential illumination. Pattern of branching in various algae and dorsiventrality in species of algae, mosses, and liverworts can be determined and in some cases reversed in later development by differential illumination. With equal illumination on both sides dorsiventrality does not appear. Gravity, centrifuging, and electric current have also been shown to be reorganizers of certain plants.[3]

REORGANIZERS IN COELENTERATE RECONSTITUTION

The demonstration by Browne (1909) that the hypostome region of *Hydra* can act as a reorganizer when transplanted to other body-levels has been repeated by various later experimenters, notably Mutz (1930 and citations) and Yao (1945). Oxidation-reduction indicators suggest a somewhat higher metabolism in the hypostome than at other body-levels (Child, unpublished). Yao finds that hypostomes exposed to certain concentrations of KCN or LiCl for 30 minutes or to NaF for 15 minutes are still capable of acting as reorganizers; and he maintains that, since these agents decrease oxidative metabolism, the reorganizing action is independent of metabolic rate. However, experiments with the concentrations and exposure periods used by Yao show that most individuals recover completely within 1–2 hours after return to water (Child, unpublished). Since Yao has not considered the possibility of recovery, his conclusion that reorganizing action is independent of metabolic rate is not justified.

Since the experiments on reconstitution in *Tubularia* by Loeb, Driesch, and Morgan, some of them more than fifty years ago, factors influencing polarity, scale of organization of the hydranth, and rate of reconstitution have been the subjects of numerous studies. Certain familiar features of reconstitution in this genus must be briefly recalled here. First, oxygen uptake decreases from the hydranth basipetally, and rate of reconstitution decreases in the same direction (Hyman, 1920, 1926; Barth, 1940*a*). Second, in pieces of *Tubularia* stems a hydranth is reconstituted at the distal cut end, and usually another, smaller and developing less rapidly, at the proximal cut end. Both hydranths develop by redifferentiation of a portion of the stem without any outgrowth from the cut end, manubrium, distal and proximal tentacles being localized at different distances from the ends of the piece. Third, hydranth reconstitution at either end of a piece can be inhibited by ligaturing that end or by sticking it in sand. Fourth, the scale of organization, that is, the length of the whole hydranth primordium and of each part of it, decreases from distal to proximal levels of section and is, in general, less in reconstitution at the proximal than at the distal cut end of a piece. It can be decreased or increased experimentally by chemical and physical agents inhibiting or increasing metabolism. Fifth, very short pieces may reconstitute only partial hydranths, complete from the apical end as far as scale of organization and length of piece or de-

[3] References in Child, 1941*a*, pp. 420, 421, 430, 431.

velopment of another partial hydranth at the other end of the piece permit. Certain extraorganismic factors are concerned in reorganizing isolated pieces of *Tubularia* and other hydroids. Constant electric current, flowing longitudinally, determines a high frequency of hydranth development at the anodal ends of pieces, and of delay or inhibition at the cathodal ends, independently of the original polarity of the piece. With much higher current densities, relations are reversed, hydranth development becoming predominantly cathodal and inhibition anodal (Barth, 1934). In *Eudendrium*, Barth found cathodal, in *Pennaria*, anodal inhibition. Hydranth development, mostly anodal, with cathodal inhibition had been reported earlier by Lund (1921) for *Obelia*. More recently it has been shown by Barth (1938, 1940*b*, 1944) and Miller (1939, 1942) that oxygen is an essential factor in tubularian reconstitution, that an oxygen gradient from the cut end must be present for hydranth development, and that with oxygenated water at the proximal end boiled water, nitrogen, or CO_2 at the distal end, hydranths develop proximally. Tubularian perisarc has been found not to be highly permeable to oxygen, and removal of a piece of lateral perisarc without section of coenosarc may result in organization and development of one or two hydranths with apical end or ends at the region of exposed perisarc (Zwilling, 1939).

In the cases of inhibition of hydranth development at one end of a piece by ligature, sticking in sand, electric current, or lack of oxygen, the extraorganismic inhibiting factors are primarily selective, rather than actual reorganizing, factors; that is, they determine the development of one of the two polarities which could develop in sea water without the external differential. However, an oxygen gradient may unquestionably act as a true primary reorganizer in Zwilling's experiments and in some of Barth's. It has not yet been shown that electric current can act in this way, but it probably can do so.

The effect of oxygen on another aspect of organization in *Tubularia* is also of interest. Barth has shown that length of hydranth increases with increase and decreases with decrease of oxygen; that is, the oxygen gradient from the cut end determines localization of distal and proximal tentacles at different distances from the cut end, and consequently length of manubrium and intertentacular region in the hydranth primordium. Similar alterations of primordium length by differences in temperature and by inhibiting chemical agents have been determined in both *Tubularia* and *Corymorpha* (Child, 1941*a*, p. 345).

As experimental material, another tubularian hydroid, *Corymorpha palma*, has the advantage of large size and absence of perisarc, except on a short basal region of the stem. Differential susceptibility to many agents, determinations of oxygen uptake, colorimetric comparative estimations of CO_2 production, and oxidation-reduction indicators all agree in showing a definite gradient pattern in *Corymorpha* with hydranth at the high end and a decrease in the proximal direction in the naked stem.[4] In stem pieces 2 cm. or more in length, lying undisturbed on one side, a hydranth usually develops at each end and one or two basal ends midway or nearly midway between the hydranths and on or near that side of the piece in contact with the glass of the container (Fig. 2, *A–C*). In such pieces oxidation-reduction gradients, with cut ends representing the high re-

[4] Child and Hyman, 1926; Child, 1926*a*; Child and Watanabe, 1935; Watanabe, 1935.

gions of hydranth-stem gradients in opposite directions, become visible before hydranths appear. The side of the piece in contact usually reduces indicators without general oxygen decrease in the water. As the small slender stolons which constitute the holdfasts develop on the basal region, each shows an oxidation-reduction gradient with high end at the tip, evidently an expression of differential growth. Doubtless the metabolism of the perisarc-secreting region or regions becomes different in character at some time from that of hydranth and stem, but before perisarc is formed a quantitative gradient pattern is present in definite relation to the reorganization of the piece.

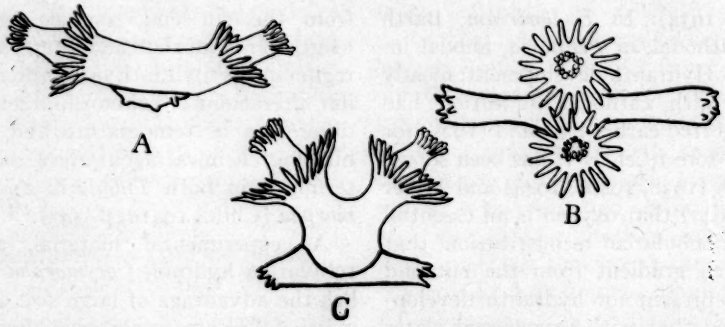

FIG. 2.—Development of bases in bipolar forms of *Corymorpha palma;* basal regions covered by perisarc indicated by heavy lines. *A*, basal region midway between the two hydranths on the side of the stem in contact; *B*, a hydranth from each end of the piece has turned upward, and two basal regions have developed from the side of the piece in contact; *C*, a hydranth has developed from the upper free side, and a base from the side in contact of each end of the piece (*A* and *C* from Child, 1941*a* and 1926*b*).

Polarity in stem pieces of *Corymorpha* can be decreased to any degree or completely obliterated by various inhibiting agents—e.g., ethyl alcohol, ethyl ether, ethyl urethane, chloretone, HCl–sea water (CO_2?)—before or after section, or both (Child, 1927*a*). During exposure to the agent the pieces lie on the side; and there is no reconstitution and no contraction and extension, such as occurs without inhibition. They become more or less flattened and apparently undergo some dedifferentiation. After return to sea water without change of position, new oxidation-reduction gradients and single, or often multiple, polarities and organizations appear at right angles to the original polar axis, with hydranth or hydranths developing on the free side and bases from the region in contact or sometimes evidently determined by developing hydranths (Fig. 3, *A–E*). The localization of the hydranths is evidently determined, in general, by the gradient between the free side and the side in contact. Since numbers of new polarities vary widely in such pieces and since they appear not only on the upper sides of the ends of pieces (Fig. 3, *A*) but in the middle (Fig. 3, *B*) or elsewhere away from the ends (Fig. 3, *D* and *E*), the position of each must be determined by slight local differences in condition, perhaps associated with groups of entodermal cells from the longitudinal entodermal canals, which lose their morphological characteristics and become cell aggregates beneath the ectoderm under the altered axial conditions. Short pieces, after temporary exposure to slightly inhibiting

conditions, which do not obliterate the original polarity but delay reconstitution and undoubtedly lower the metabolic level of the region in contact, often develop a permanent dorsiventrality after return to sea water (Fig. 3, *F–H*).

Cells from *Corymorpha* stems dissociated by pressing through fine bolting cloth undergo aggregation. In small cell aggregates allowed to remain undisturbed on the bottom of the container, development of partial hydranths or of complete individuals occurs with apical ends at or near the upper free surface and bases from the region in contact. Cell aggregates kept from contact by slow revolution in closed containers on a vertical wheel usually remain spherical, do not develop at all, gradually decrease in size, and before death may become completely surrounded by perisarc. Occasionally, however, polarity and de-

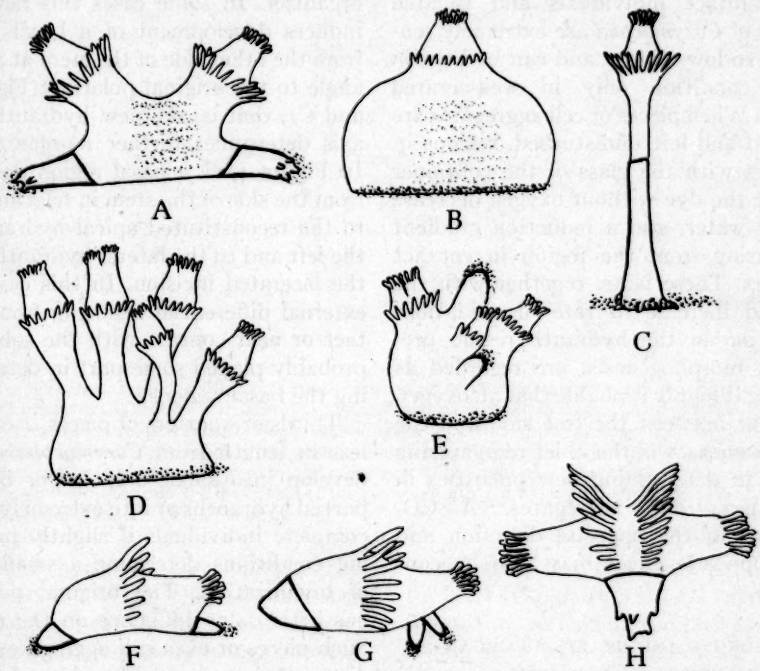

FIG. 3.—Reorganization with determination of new axiate patterns in *Corymorpha* pieces by the external differential between the free side of the piece and the side in contact after decrease or obliteration of the original polarity by differential inhibition; the delicate perisarc often secreted in early stages of the new basal regions and displaced by the usual type of perisarc is indicated by stippling. *A–C*, ethyl alcohol 2 per cent for 48 hours following section; development in water. *A*, a hydranth and a base from each end of the piece, old longitudinal entodermal canals still visible in middle region; *B*, a single hydranth from the upper side and a basal region developing from the side in contact, longitudinal entodermal canals still visible in the middle region; *C*, a later stage of *B* after complete reorganization. *D*, ethyl ether 0.25 per cent for 26 hours preceding section, ends of piece right and left, five new axes reorganized from free side, base developing from side in contact; *E*, chloretone 0.02 per cent for 26 hours following section, four hydranths, one with two manubria, two bases, apparently organized by the hydranths, all from the free side, and another base developing from the side in contact; *F*, *G*, *H*, permanent dorsiventral patterns organized by the free-surface-contact differential after slight inhibition, followed by return to water (from Child, 1941*a* and 1927*a*).

velopment appear in such aggregates, presumably because a few cells at a higher level of activity than others initiate a gradient, that is, act as organizers (Child, 1928, 1941*a*, p. 419). It is to be emphasized that in all these stem pieces and aggregates it is not an extraorganismic factor as such—whether oxygen, CO_2, or something else—but the differential, the gradient, in its action, which is the organizer or the reorganizer. Both intact individuals and isolated pieces of *Corymorpha* are extremely sensitive to low oxygen and can be kept in good condition only in well-aerated water. When pieces or cell aggregates are stained and left undisturbed, regions in contact with the glass of the container reduce the dye without oxygen decrease in the water, and a reduction gradient decreasing from the region in contact appears. These facts, together with the marked increase in rate of oxidation-reduction in the hydranth region preceding morphogenesis, are regarded as making it highly probable that an oxygen gradient between the free side and the side in contact is the chief reorganizing factor in determining new polarities in the pieces and aggregates. A CO_2 gradient in the opposite direction and with opposite effect may also be concerned.

INCISIONS AND GRAFTS AS ORGANIZERS IN *Corymorpha*

Under natural conditions dominance of the apical hydranth prevents formation of hydranth buds in *Corymorpha*. Smooth transverse lateral incisions, even halfway through the stem, usually close in a few hours, with no development if the apical hydranth is present and in good condition. If the hydranth is removed when the incision is made, hydranth development after closure of the incision occurs somewhat more frequently. Closure of a lacerated incision made by numerous radiating cuts (Fig. 4, *A*) is much less rapid; and if the apical hydranth is removed a lateral hydranth-stem axis frequently (43 per cent) develops after complete closure of the incision while the apical hydranth is undergoing reconstitution (Child, 1927*b*, 1941*a*, pp. 371, 372). The cells activated by the lacerated incision become a reorganizer. In some cases this new axis induces development of a basal region from the other side of the stem at a right angle to the original polarity (Fig. 4, *B* and *C*); that is, the new hydranth-stem axis determines further reorganization. In Figure 4, *C*, a basal region develops from the side of the stem in relation both to the reconstituted apical hydranth at the left and to the lateral hydranth from the lacerated incision. In this case, the external differential resulting from contact or near contact with the substrate probably played some part in determining the basal regions.[5]

Thirds or quarters of pieces, 1 mm. or less in length from *Corymorpha* stems, develop into small unipolar or bipolar partial hydranths or into extremely small complete individuals if slightly inhibiting conditions determine a small scale of organization. The original polarity has little or no influence on the result. Such pieces or even cell aggregates from dissociated stems, used as lateral grafts in small incisions in the stems of host individuals, can induce new polarities, giving rise to hydranth-stem axes in which they form only the distal part of

[5] Local laceration of corresponding regions of distal and proximal cut ends of the body wall in pieces of the actinian *Harenactis* and their later union in "rings" result in a wide range of degrees of development of new polarities and of radial and bilateral symmetries, and in some cases in development of normal axiate patterns (Child, 1941*a*, pp. 373, 374).

the manubrium of the new hydranth (Fig. 5, *A* and *B*). The frequency of this induction is higher when the apical hydranth of the host is removed at the time of implantation. Pieces from distal stem levels, that is, higher gradient-levels, are more effective than those from lower gradient-levels of the proximal region. Implants in the region of reconstitution of the apical hydranth may induce manubrium or even more or less in good condition, usually inhibits development of a hydranth there and often induces development of a basal end instead of a hydranth. When a piece that includes the induced lateral hydranth and several centimeters of the host stem is isolated, a basal region, instead of a hydranth, often develops at both distal and proximal ends of the host stem piece (Fig. 5, *F*). In these cases the induced new axis completely reverses the polarity

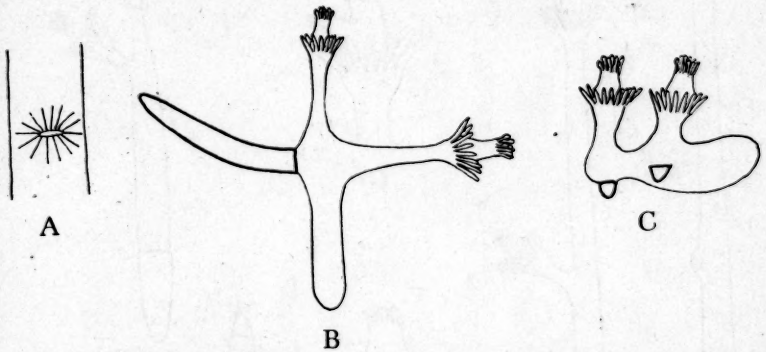

FIG. 4.—Reorganization by outgrowth after closure of a lacerated lateral incision in the *Corymorpha* stem. *A*, the character of the incision with lacerated edges; *B*, hydranth developing from lacerated incision at right, apical reconstituted hydranth above, somewhat inhibited by lateral hydranth, basal region at left, reorganized at right angle to the original polarity by the hydranth from the incision; *C*, apical hydranth reconstituted at left of figure, lateral hydranth from lacerated incision to the right of it, basal region reorganized in relation to each hydranth from the lower side of the piece and at right angle to the original polarity (*B* from Child, 1941*a* and 1927*b*; *C* from Child, 1927*b*).

complete hydranth development (Fig. 5, *C* and *D*). When the induced lateral hydranth is near the reconstituted distal hydranth, it frequently brings about decrease in size and regression of the distal hydranth and stem (Fig. 5, *E*) and finally their complete disintegration, so that it becomes the distal hydranth. In these cases it becomes a disorganizer of the regions distal to it (Child, 1929*a*, 1932, 1941*a*, pp. 378–81). The physiological basis of this disorganizing action becomes evident from other experiments. If the stem is cut off 1–2 cm. distal to the induced lateral hydranth, this hydranth, if of the stem for some distance distal to its level (Child, 1935). Less extreme reorganizing actions on the host stem consist in retardation and decrease in scale of organization or complete inhibition of the distal hydranth, with no reorganization of a base but often with a base at the proximal end, in place of the hydranth usually developing there in isolated stem pieces or sometimes merely in absence of any reorganization at either end of the host stem. Reduction and disintegration of host hydranth and stem distal to the induced lateral hydranth (Fig. 5, *E*) evidently result from the re-

organizing action of the new lateral axis on regions of the host distal to it.[6]

OBLITERATION AND INDUCTION OF POLARITY IN EMBRYONIC HYDROZOAN DEVELOPMENT

The oöcytes of the hydrozoan *Phialidium gregarium* form a columnar epithelium along the radial canals of the medusa, with one pole of each cell attached to the wall of the canal, the other separated from the water by only a very delicate membrane, covering the gonad (Fig. 6, *A*). In oöcytes of various stages removed from the gonad, but still showing the region of attachment, differential susceptibility and rate of reduction decrease from the pole adjoining the membrane, and this becomes the apical pole (Fig. 6, *B*, *C*, *D*). This polar gradient persists to planula stages but can be decreased to any desired degree or completely obliterated by differential inhibition resulting from differential susceptibility to various agents, e.g., KCN, LiCl, ethyl urethane, HCl–sea water (probably CO_2), and by presence of large numbers of the medusae (probably CO_2). In normal development cells immigrate from the lower end of the polar gradient and form entoderm (Fig. 7,

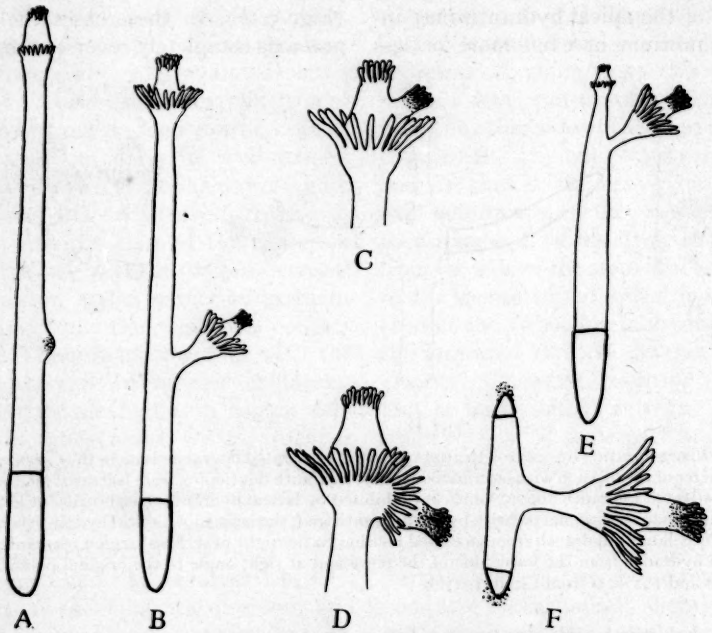

FIG. 5.—Lateral grafts of very small stem pieces of *Corymorpha* as reorganizers; the region developing from the graft approximately indicated by stippling. *A*, early stage of outgrowth; *B*, induced lateral and reconstituted distal hydranth; *C*, *D*, induction by grafts in region of distal hydranth reconstitution; *E*, inhibition and reduction of stem and hydranth distal to induced lateral hydranth; *F*, induction of basal regions at distal and proximal ends of a stem piece by a lateral hydranth induced by a graft. (*A–D* after Child, 1932 and 1935; *E* and *F* not previously published.)

[6] For further data and references on hydroid reconstitution see: Child, 1941*a*, pp. 314–19, 333–35, 344–49, 359–61, 371–74, 378–81, 413–20.

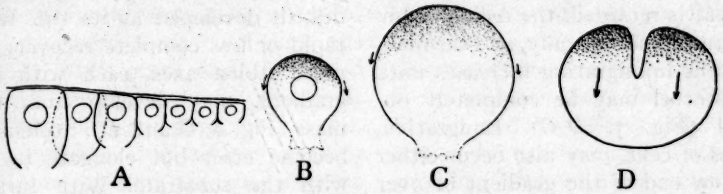

FIG. 6.—Oöcytes and first cleavage of hydrozoan *Phialidium gregarium*. *A*, part of gonad with oöcytes forming columnar epithelium, each with one pole attached to wall of radial canal and opposite pole separated from sea water by a thin membrane. *B*, *C*, reduction gradient from free pole of early and advanced oöcyte; *D*, first cleavage furrow and reduction gradient (from Child, 1941*a* and 1925*a*).

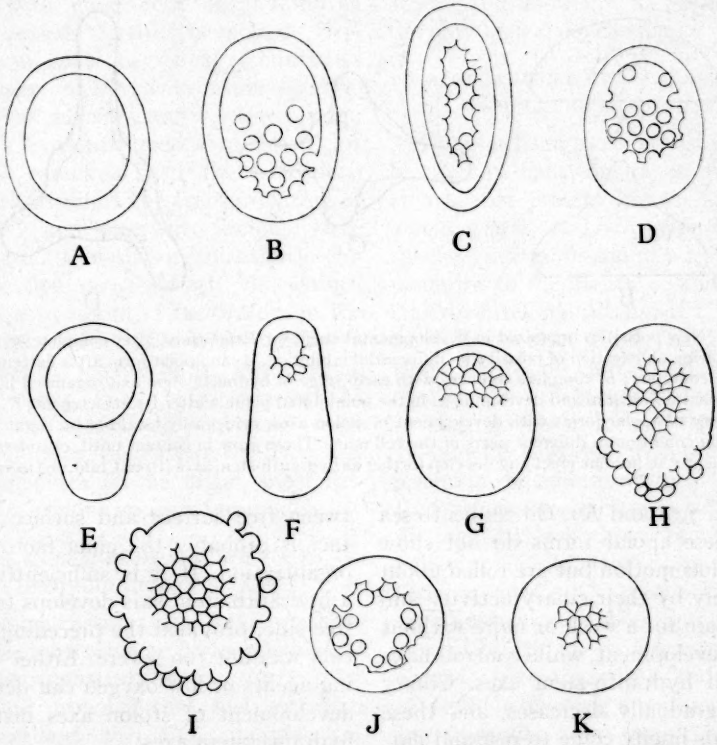

FIG. 7.—Normal and differentially inhibited development of *Phialidium*. *A*, *B*, *C*, normal development, showing immigration of cells from "vegetal" pole and formation of entoderm from them; *D*, *E*, *F*, stages and degrees of nonspecific differential inhibition by various external agents, showing increase in number of immigrating cells and extension apically of region of immigration; *G*, *H*, *I*, obliteration of blastocoel by immigrating cells and occurrence of emigration; *J*, apolar form with immigration from all regions of cell wall; *K*, apolar form, blastocoel obliterated (from Child, 1941*a* and 1925*b*).

A–C). With differential inhibition development is retarded, the region of immigration extends apically, and the number of cells immigrating increases until the blastocoel may be completely obliterated (Fig. 7, *D–G*). Emigration, with loss of cells, may also occur either at the low end of the gradient or over most or all of the surface (Fig. 7, *H* and *I*). Individuals in which polarity is obliterated become completely spherical, and immigration occurs at all levels, with complete obliteration of the blastocoel (Fig. 7, *J* and *K*). On return to sea water these apolar forms do not show directed locomotion but are rolled about indefinitely by their ciliary activity and may remain for a week or more without further development, while controls have developed hydranth-stem axes. Ciliary activity gradually decreases, and these individuals finally come to rest and flatten somewhat at the bottom of the container (Fig. 8, *A*). In well-aerated water many recover to such an extent that a hydranth-stem axis develops from the free surface. In the individual shown in Figure 8, *B*, the outgrowth from the upper side continued to elongate and a hydranth developed at its tip. With less rapid or less complete recovery, one or more stolon axes, each with its own gradient, may develop from the cell mass (Fig. 8, *C* and *D*). Stolons do not become erect but elongate in contact with the substrate. With further recovery the stolon tip may become a hydranth-stem axis and turn away from the substrate. In this case, as in the differentially inhibited pieces of *Corymorpha* stem, an oxygen gradient between free surface and surface in contact is probably the chief factor in reorganization. If it is sufficiently steep, a hydranth-stem axis develops from the free side, provided the preceding inhibition was not too severe. Either inhibiting agents or low oxygen can determine development of stolon axes instead of hydranth-stem axes.

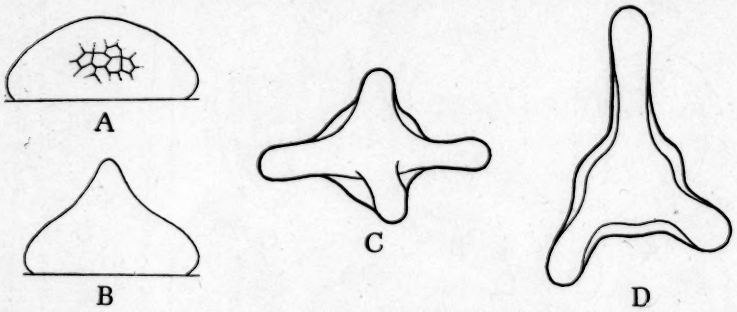

FIG. 8.—New polarities organized in developmental stages of *Phialidium*, after complete or partial recovery following obliteration of polarity by differential inhibition. *A*, an apolar form after flattening on the glass of the container; *B*, complete recovery with early stage of hydranth-stem axis organized by the free-surface-contact differential and developing as in the uninhibited planula after its attachment; *C*, *D*, incomplete recovery of apolar forms with development of stolon axes, evidentally localized by slight fortuitous differences in condition in different parts of the cell mass. These grow in contact until, or unless, they recover sufficiently to become erect and develop further as hydranth-stem axes (from Child, 1941*a* and 1925*b*).

It has been demonstrated for a number of hydroid species that stolon axes develop from slightly inhibited gradients. Apical regions of hydranth-stem axes have been transformed into stolon axes by slight inhibition with chemical agents

and in some species by removal from natural conditions to standing water for 24 hours. Under these conditions the hydranths disintegrate and the apical regions of the stems begin growth as stolons (Child, 1923, 1941*a*, pp. 172–75). With transfer to well-aerated running water, hydranth-stem axes may develop from the stolon tips. In less sensitive species low concentrations of KCN, anesthetics, and other agents induce the transformation of hydranth-stem axes into stolon axes; and on return to sea water with good aeration, hydranths again develop from stolon tips. Evidences of some degree of acclimation have been observed in some species, consisting in appearance, after some days, of hydranth buds from stolon tips without removal from the conditions which determined the transformation of hydranth-stem axes into stolons. Here the character of the organization or reorganization is apparently determined by oxygen content of the water, on the one hand, and by differential inhibition by chemical agents or by low oxygen, on the other. That the oxygen and the inhibiting agents do anything more primarily than increase or decrease rate of metabolism in the distal, most susceptible, regions appears improbable.

THE QUESTION OF SPECIFICITY IN HYDROID REORGANIZERS

In some cases oxygen gradients have been demonstrated to be reorganizers in hydroid reconstitution; in other cases it is highly probable that such gradients and perhaps also CO_2 gradients reorganize. These are extraorganismic reorganizers. Hydranth primordia, hydranths, implants of very small stem pieces or cell aggregates, and the cell activities resulting from lacerated incisions are intraorganismic reorganizers. Both kinds can induce new polarities, and there is no evidence that any of them are specific in action or essentially different from others. Moreover, there is no evidence that the action of low oxygen and of differentially inhibiting agents in transforming hydranth-stem axes into stolon axis is specific rather than quantitative. In view of the experimental data, the conclusion that these reorganizing actions are not specific in character does not appear to be "a counsel of despair" (p. 121). On the other hand, it seems, at present, to be the only logical conclusion.

REORGANIZERS IN PLANARIAN AND ANNELID RECONSTITUTION

The planarian species most extensively used in experiments on reconstitution are at present known as *Dugesia dorotocephala* and *D. tigrina*.[7] Both species undergo fission at a level slightly posterior to the mouth or under certain experimental conditions, at a more posterior level also. Under natural conditions there is no morphological evidence that the posterior region consists of one or more zooids in an early stage of development. However, with prevention of fission in the laboratory by slow growth, the posterior zooid often becomes clearly distinguishable both by its form and its behavior (Child, 1941*a*, pp. 321–23). Oxygen uptake, CO_2 production, differential reduction of dyes, and differential susceptibility to a large number of agents, both chemical and physical, all agree in showing a gradient, decreasing from the head posteriorly to the fission zone, with an increase in the posterior zooid.[8] In planarian species that

[7] This genus was earlier known as *Planaria*, then as *Euplanaria*. See Child, 1941*a*, p. 42 n.

[8] Child, 1941*a*, pp. 108–17, 181–90, 321–23. Even the CO_2 determinations by Parker (1929),

do not undergo fission, the gradients decrease over the whole length, except for a slight increase at the extreme posterior end, apparently a region of growth in length.

Although these planarians differ widely from *Tubularia* and *Corymorpha* in structure and physiology, there is a very interesting similarity in various features of reconstitution of isolated pieces which suggests that the factors concerned are, in considerable degree, independent of the specific constitutions of hydroid and planarian protoplasms. Except at extreme anterior body-levels, the regenerating planarian head, like the hydranth, is "out of place." In pieces from other levels reconstitution does not consist in regeneration of the region originally between the anterior end of the piece and the head of the intact animal, with head formation last of all. On the contrary, head regeneration begins first at the cut surface; and reorganization from the old tissues or regeneration of parts between the head and the original body-level of the piece follows. In a piece representing the prepharyngeal region, except the head, the region *M* of Figure 9, *A*, the head is larger and develops more rapidly, and the new pharynx is localized farther posteriorly, than in a piece representing the region *N* of Figure 9, *A*, as Figure 9, *B* and *C*, indicate. The latter piece includes the fission zone (region *F*, Fig. 9, *A*) and the anterior region of the posterior zooid, but the reorganization of the piece obliterates these. The reorganizing action of the

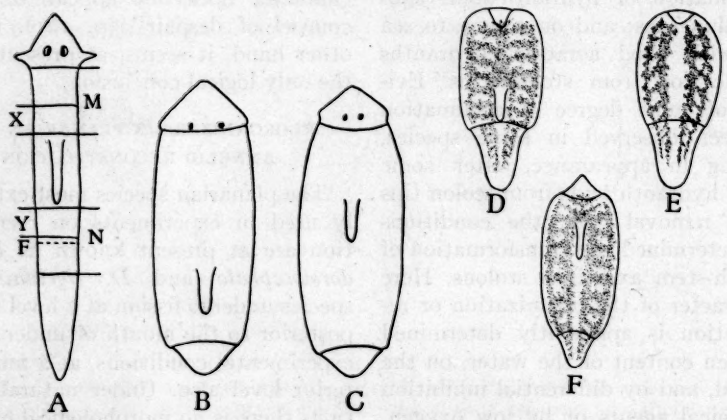

FIG. 9.—Planarian reconstitution. *A*, diagrammatic outline indicating levels of section and approximate position of fission zone, *F; B*, stage of reconstitution of the long prepharyngeal piece, *M*, with pharynx localized near the posterior end and short postpharyngeal region; *C*, stage of reconstitution of the long postpharyngeal piece, *N*, with head regenerating at the anterior cut surface and induction of prepharyngeal and pharyngeal region in the anterior part of the piece; *D*, a prepharyngeal acephalic form from region *X* with reorganization of regions posterior to the body-level from which it was taken; *E*, a postpharyngeal acephalic form from the region *Y* with reorganization of regions posterior to its level of origin, but nothing anterior to this level; *F*, a postpharyngeal piece from the region *Y* on which an inhibited anophthalmic head has regenerated and has induced reorganization of a prepharyngeal and pharyngeal region (*D*, *E*, *F*, from Child, 1941*a* and 1929*b*).

which he regarded as showing absence of a gradient, actually show the same gradient pattern as the other lines of evidence. Parker either ignored or was unaware of the presence of a posterior zooid.

regenerating head is more clearly indicated in the posterior than in the anterior piece. The posterior piece came from a level posterior to the pharynx, but in reconstitution a pharynx and a prepharyngeal region are reorganized in it. However, this case merely suggests but does not prove that the reorganization is dependent on the regenerating head or its blastema.

Within a certain range of length of piece in relation to length of the original animal and body-level from which the piece was taken, the stimulation resulting from section at the posterior end of the piece inhibits head development differentially, median regions being more susceptible to the inhibiting conditions than lateral. Different degrees of this inhibition, according to body-level and length of piece, determine a graded series of head forms, ranging from the normal through the forms designated as teratophthalmic, teratomorphic, and anophthalmic, to completely acephalic. In pieces of equal length this differential inhibition of head regeneration increases in degree and frequency from anterior body-levels to the fission zone and decreases in the posterior zooid region (Child, 1941*a*, pp. 177–87). Delay of posterior section for even an hour or two after anterior section decreases the inhibiting action (Child, 1941*a*, pp. 406–10). Since section of the nerve cords at certain distances from the anterior end of the piece is almost as effective as complete transverse section of the body, the differentially inhibiting physiological factor is regarded as largely or entirely nervous in character. It is only temporary, disappearing some hours after section. There is also a temporary increase in oxygen uptake and CO_2 production following section, greater in shorter than in longer pieces, like the factor inhibiting head regeneration (Hyman, 1923; Robbins and Child, 1920). In different planarian species physiological inhibition of head regeneration appears in different degrees—in some species not at all. Apparently, either activation of the head-forming cells is sufficient in some species to overcome any inhibiting action or the nervous stimuli resulting from posterior section are less intense.

Certain ranges of concentration or dosage of various chemical and physical agents also inhibit head regeneration differentially, producing exactly the same graded series of head forms as the physiological inhibiting factor. Moreover, the same agent may decrease or increase the frequencies of the inhibited head forms, according as it is used for a short time after section to inhibit the physiological inhibiting factor or during the whole period of head regeneration to inhibit the head-forming cells. Also, a single concentration of the same agent may inhibit normal head regeneration in anterior pieces, where it is high in frequency, and increase it in more posterior pieces, where it is low. High temperature, increased motor activity of pieces, and abundant food preceding section decrease the frequencies of differentially inhibited heads; low temperature, decrease of motor activity, and starvation increase the frequencies of the differentially inhibited forms by action on the head-forming cells.[9]

In pieces of certain lengths, relative to total length of the original animal, and from the more posterior regions of the anterior zooid (e.g., pieces *X* and *Y* of Fig. 9, *A*, and similar intervening pieces) completely acephalic forms usually result from the inhibiting effect of pos-

[9] For further data, interpretations, and references see Child, 1941*a*, pp. 181–96.

terior section (Fig. 9, *D* and *E*). However, pharynx and mouth are reorganized in acephalic forms from levels anterior to the pharynx (e.g., Fig. 9, *D*, from region *X*, Fig. 9, *A*). In acephalic forms from levels posterior to the pharynx and mouth (region *Y*, Fig. 9, *A*). there is no reorganization of body-levels anterior to the body-level from which the piece was taken (Fig. 9, *E*.) But if the merest rudiment of a head regenerates—even a completely anophthalmic head in which only a few ganglionic cells are present—mouth, pharynx, and a short prepharyngeal region are formed (Fig. 9, *F*). Extensive experiment with headless pieces has shown that reconstitution of body-levels anterior to the region of the original animal from which the piece was taken never occurs unless or until there is some degree of head development at the anterior end. On the other hand, reconstitution of body-levels posterior to that from which the piece was taken occurs in complete absence of head regeneration in pieces from any level (Fig. 9, *D* and *E*). If the anterior end of an acephalic piece, such as Figure 9, *E*, is removed after the posterior end has developed, a normal head regenerates and reorganization of mouth, pharynx, and prepharyngeal region occur; but if section is made posteriorly in the piece at the same time as anterior section, it again becomes an acephalic form.

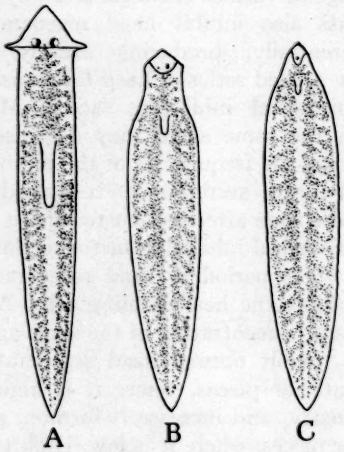

FIG. 10.—*A–C*, decrease in scale of organization of prepharyngeal and pharyngeal region, with inhibition of head development by external agents (from Child, 1941*a* and 1929*b*).

The regenerating head or the active head blastema is the reorganizer of body-levels anterior to the level of the piece in the original animal. Differential inhibition of head development decreases its effectiveness as reorganizer. Differences in scale of organization of pharyngeal and prepharyngeal regions with uninhibited and inhibited head development are shown in Figure 10, *A–C*.[10]

In pieces so short that the fraction of the polar gradient present is so small that it has little or no determining effect, unipolar or bipolar partial forms develop, consisting of heads only or of heads with more or less of the post-cephalic regions, the character of development depending on length of piece, body-level from which it was taken, physiological condition of the animal, and external medium. In such pieces, taken from near the anterior end of the body, the partial forms are usually unipolar (Fig. 11, *A–C*). In pieces from regions at or near the fission zone, where the change from the low gradient-level of the posterior end of the anterior zooid to the higher level of the anterior region of the posterior zooid occurs, the two ends may be almost alike, and bipolar forms occur in relatively high frequency (Fig. 11, *D–I*). In these bipolar forms

[10] For more recent experiments on alteration of scale of organization see Rulon and Child, 1937; Rulon, 1944.

one or two posterior ends are sometimes induced laterally (Fig. 11, *E* and *F*). These cases resemble some of the inductions of basal regions in bipolar forms of *Corymorpha* (see Fig. 2, *B*). Frequency of planarian bipolarity can be considerably increased by exposure to inhibiting agents for a time immediately following section, in order to decrease still further, by differential inhibition, whatever polar gradient may still be present, with return to water before appreciable velopment of a dorsal outgrowth (Fig. 11, *K*)[11]

The most striking evidence of reorganizing action by the planarian head region is provided by implants of small pieces, including a part of the cephalic ganglia. Such pieces may, when implanted in the body of another individual, regenerate a complete normal head or variously modified head structures and may induce development from host tissues of postcephalic regions of different

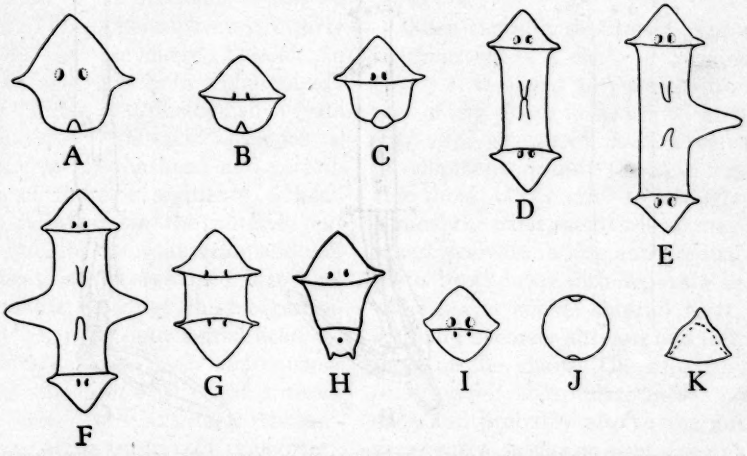

FIG. 11.—Unipolar and bipolar partial forms and apparently apolar forms in planarian reconstitution. *A*, *B*, *C*, unipolar partial axes; *D*, *E*, *F*, bipolar, primarily partial axes with secondary development of one or two posterior ends in *E* and *F*; *G*, *H*, *I*, bipolar partial axes; *J*, *K*, dorsal and sectional outline of an apparently apolar form (from Child, 1941*a*).

head regeneration occurs (Rustia, 1925). Extremely short planarian pieces often appear to be completely apolar (Fig. 11, *J*); they show no regeneration from either end, become circular in outline, new tissue merely fills in the contracted cut ends, and directed locomotion is absent. The longitudinal muscles become almost circular; and when they contract, the piece becomes concave ventrally, resembling a contracting medusa, and the central regions are forced dorsally, often with gradual delength and morphology, according to level of implantation and other experimental conditions. This reorganizing action of cephalic implants is not species-specific; it occurs between *Dugesia dorotocephala* and *D. tigrina* with either species as host or graft. For the great variety of forms resulting from the reorganizing action of the cephalic implants the original papers must be con-

[11] Further data on bipolar and multipolar forms and on origins of new polarities in Child, 1941*a*, pp. 364–67.

sulted.[12] Here, only one type of reorganization need be considered. Cephalic grafts implanted in the posterior zooid, where polarity and organization are more labile than elsewhere, because of the changes occurring in the gradual development of the zooid, give rise to a head which may be normal if one side of the graft remains free and regenerates. This head induces development of a postcephalic region from host tissue; it is covered externally with either dorsal or ventral ectoderm, according as it grows out from the dorsal or ventral surface of the host (Fig. 12, *A*). If the graft takes on all sides, it develops as a rounded outgrowth with varying numbers of photoreceptors, and this induces a further cylindrical outgrowth from the host. In this case the induced outgrowth is usually or always hollow, with dorsal ectoderm outside, ventral ectoderm inside, if it grows out from the dorsal surface, and with ventral ectoderm outside, dorsal ectoderm inside, if it grows out ventrally (Fig. 12, *B* and *C*). A further reorganization of the host body occurs posterior to the graft and also anterior to it for a variable distance with complete reversal of polarity, so that finally three polarities are present (Fig. 12). The reversal of polarity anterior to the graft occurs not all at once but gradually. If the host body is sectioned at the level *XX* of Figure 12, *B*, soon after implantation, a normal head develops on the posterior piece containing the graft. After removal of the normal head a differentially inhibited head may develop; but finally, after reversal of polarity is completed to the level *XX*, a

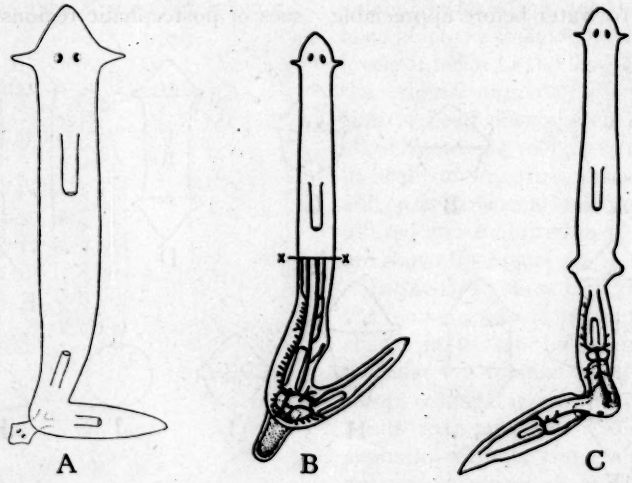

FIG. 12.—Heteroplastic grafts of small pieces, including part of the cephalic ganglia, from *Dugesia tigrina novangliae* to the postpharyngeal region of *D. dorotocephala;* reorganization both posterior and anterior to the anterior region induced by the graft, with complete reversal of polarity for a considerable distance anteriorly; in *B* and *C* the grafts are effective reorganizers, although the heads developing from them are abnormal and more or less rudimentary (from Child, 1941*a; A* originally from Santos, 1929, 1931; *B* and *C* from Miller, 1938).

[12] The more recent papers are: Santos, 1931; Okada and Sugino, 1937; Miller, 1938. Discussion and references to other work in Child, 1941*a*, pp. 381–87.

posterior end, instead of a head, regenerates, and the posterior piece containing the graft becomes an individual with two posterior ends and the graft head as its head. Moreover, the two posterior ends may gradually fuse into one, though retaining the double intestinal pattern. The reversal of polarity anterior to the graft head is similar to the reversal by the lateral graft-induced hydranth in *Corymorpha* (Fig. 5, *F*).

In annelid reconstitution the regenerating head or its blastema is also an organizer. This is perhaps most clearly shown in the polychete *Sabella*, in which the more posterior "abdominal" segments differ morphologically from more anterior "thoracic" segments. After removal of the head and certain numbers of thoracic segments, a head may regenerate from the anterior cut surface, and the more anterior abdominal segments are reorganized into thoracic segments. However, this reorganization may occur without actual head development, apparently in consequence of the activation of cells at the anterior cut end. Some degree of similar reorganization may occur anterior to the posterior cut end, recalling reorganizing action of hydranths developing at the proximal ends of pieces and planarian heads developing from posterior cut ends.[13]

In the earthworm and various other oligochetes the regenerating head region or its blastema reorganizes a region posterior to it. Like the hydranths of *Tubularia* and *Corymorpha* and the planarian head in reconstitution, it is "out of place" except when the cut end is immediately posterior to the original head. In *Lumbriculus*, as in the planarian, head regeneration may be more or less inhibited in short pieces by the stimulation from the posterior cut end (Hyman, 1916).

Regeneration of a head slightly anterior to the physiologically distinguishable planarian fission zone or an early visible fission plane in *Stenostomum* and aquatic oligochetes may induce regression of the fission zone and reorganization of the very early stages of head development posterior to it into postcephalic levels of the individual reorganized by the regenerating head or its blastema.

When the fully developed head at the anterior end of a chain of *Stenostomum* zooids is removed and a piece from the rest of the chain is sectioned in such a way that the zooid most advanced in development, or only the head region of this zooid, is posterior to less advanced zooids, the older zooid or head may bring about decrease in size and gradual complete breakdown into separate cells of the younger zooids anterior to it, until it finally becomes anterior and forms the head of the chain. The cells from the other zooids accumulate in the pseudocoel, and probably also in the gut, and apparently serve as nutritive material for the growth of the dominant zooid. In this case the head of the older zooid acts as a disorganizer on the earlier zooids anterior to it.[14]

THE INDEPENDENCE OF HYDRANTH AND HEAD IN RECONSTITUTION OF *Tubularia*, *Corymorpha*, PLANARIANS, AND ANNELIDS

The hydranth developing at the distal end of a stem piece of *Tubularia* or *Corymorpha* is, as already noted, "out of place," except when section is immediately proximal to the original hydranth. At other levels there is no re-

[13] For further data see Berrill, 1931; Berrill and Mees, 1936*a*, *b*; Gross and Huxley, 1935.

[14] Child, 1903, 1941*a*, pp. 399–406; Van Cleave, 1929.

constitution, preceding hydranth development, of regions originally distal to the level of section. Hydranth reconstitution occurs or begins first after section; and elongation and reorganization of the stem proximal to it follow, instead of preceding, and do not occur unless the hydranth develops. Hydranth development at the proximal end of a piece is obviously out of place. This has been recognized by calling these cases "heteromorphosis." Actually, however, the distal hydranth at any other than extreme distal levels is also a heteromorphosis. Moreover, from short stem pieces partial hydranths develop, completely normal distally but lacking proximal parts in all degrees until they consist of only tips of manubria. Hydranth organization is evidently independent of the rest of the piece, and distal regions of the hydranth develop independently of proximal regions. In other words, reconstitution in *Tubularia* and *Corymorpha* is a one-way developmental gradient from the cut end or ends of the piece, and in the naked stem of *Corymorpha* it is preceded by a great increase in rate of dye reduction, also in a one-way gradient in the hydranth region. This gradient is established within a few hours after section and long before any other evidence of hydranth development appears (Child and Watanabe, 1935; Watanabe, 1935). In *Tubularia* conditions are undoubtedly similar, but hydranth reconstitution occurs inside the perisarc, so that oxidation-reduction indicators are of little use. The induction and reorganization by grafts of small stem pieces of *Corymorpha* undergoing reconstitution, and of the hydranths induced by them, constitute further evidence of one-way dominance and gradient pattern. All the evidence available indicates that the inducing and reorganizing action of the grafts, of hydranth primordia, and of hydranths is dependent on their relatively high level of metabolism. This high metabolic level makes it possible for them to alter and determine lower gradient levels. In short, the stem piece does not regenerate missing parts in succession and finally a hydranth. The region of high metabolism which becomes a hydranth, or the small piece which becomes the apical end of a hydranth, reorganizes the piece. Apparently, the only action that other parts of the piece can exert on the developing hydranth is inhibitory. With decreasing length of piece in *Tubularia mesembryanthemum* the hydranth primordium decreases in length while the length of piece is still considerably greater than the primordium length. It is evidently inhibited by the activation and induction at the proximal end of the piece following section.

The regenerating planarian and annelid head are, like the hydranth, "out of place" and "heteromorphic" when they develop at any other than an extreme anterior level. The planarian piece does not regenerate a head; the head or the activated, head-forming blastema forms independently of the rest of the piece and reorganizes a smaller or larger part of it into regions anterior to its level of origin in the original body. Heads with fully developed cephalic ganglia can form in pieces of the nemertean *Lineus* (Coe, 1932, 1934*a*, *b*) and also in annelids, in complete independence of the nerve cord. In the earthworm a head can regenerate when the nerve cord is removed or turned back from several segments posterior to the level of section. In the regenerating planarian head independence of the ganglia is less easily demonstrated because of their nearness to the old nerve cords, but has been re-

ported.[15] In so far as other parts of the piece have any effect on hydranth or head regeneration, the effect is inhibitory. Hydranth and head develop "in spite of" the rest of the piece. Hydranths or partial hydranths and planarian heads can develop in absence of other parts. This is true of hydranths to a more extreme degree than of heads; even the extreme apical hydranth regions can develop normally in complete absence of other parts. The hydranth and partial hydranth and the head are independent differentiations in reconstitution, so far as other parts of the piece are concerned. They are the morphological expressions of what may be called the "primary action system" of the species. Every body-level of *Tubularia*, *Corymorpha*, and various planarians and annelids can develop as hydranth or head if it is free from the reorganizing action of a higher gradient-level and not inhibited by extraorganismic factors. In order to develop as hydranth or head, the cells concerned must attain a certain metabolic level relative to other parts. In the tubularian hydroid this level is almost always attained unless extraorganismic factors inhibit. In the planarian, failure to attain this level, whether through the action of physiological or extraorganismic factors, results in mediolateral differential inhibition of head development, the different degrees of inhibition constituting a graded series from normal heads to complete acephaly. Visible localization of the hydranth region as a region of increased rate of oxidation-reduction within a few hours after section and activation of the planarian head blastema constitute the first distinguishable steps in development of a new individual which is independent of the old organization. Further progress of this development consists in differentiation of hydranth or head and reorganization of old parts of the piece and perhaps also of regeneration of basal or posterior parts under the dominance of higher gradient-levels. Experimental evidence indicates that the blastema developing from the cut surface of the regenerating amphibian leg is a somewhat similar reorganizer or dominant region.

Certainly, dependent differentiation is not at the heart of this form of development (p. 122); it is secondary to independent differentiation. Dependent differentiation must be dependent on something. Independent differentiation is only independent of other parts of the individual; obviously, it is a feature of a spatial pattern which must have been determined in some way. Is not the fundamental problem of development the problem of the origin and nature of the pattern, the organization which makes possible both independent and dependent differentiation?

As regards the question how the apical region of the hydranth primordium and the planarian and annelid head or its blastema exercise their reorganizing action "at a distance" in determining the localization of parts at different distances from themselves, the evidence points to certain conclusions. These reorganizing regions alter gradient patterns in the regions concerned. The hydranth primordium and the head raise gradient-levels; that is, they make the parts more apical or more anterior as the first distinguishable step in their reorganization. The distances from the reorganizers of particular localizations can be altered experimentally in either direction by external conditions which inhibit or accelerate metabolism; that is, the

[15] References on independent regeneration of ganglia in Child, 1941*a*, pp. 338–40.

scale of their reorganizing action can be decreased or increased. Reorganization of the nervous system, progressing from the apical region of the developing hydranth and from the new cephalic ganglia of the regenerating head, is, of course, a feature of the reorganization; but we do not know at present what part it may play in the differentiation at different levels of reorganization.

Reconstitutional development in the forms under discussion differs widely from the somewhat speculative theory of development recently advanced by Holmes (1944). In this theory balance and homeostasis are regarded as playing important roles. In these reconstitutions there is no balance of parts but a one-way dominance, an independence of certain regions, and their reorganizing action on others. Moreover, it seems somewhat difficult to find valid evidence of homeostasis in this or in other forms of development, characterized, as they are, by progressive physiological and morphological change and progress from one stage to another.

IS THERE AN ORGANIZER IN THE POSTERIOR REGION OF THE INSECT EGG?

Experiments with ligature, local cautery, and ultraviolet radiation of early developmental stages of the libellulid *Platycnemis pennipes* led Seidel[16] to postulate a "Bildungszentrum," that is, an organizer in the posterior region of the egg. Complete separation by ligature or killing the posterior region in stages after nuclei have migrated to the egg surface, but before blastoderm formation, prevents formation of the embryo but not further cell multiplication without embryonic pattern. Seidel concludes that a substance necessary for development passes anteriorly from the posterior region and that its passage is blocked or it is destroyed by the experimental procedures. This postulated developmental pattern not only differs from any pattern known in other forms but assumes the existence and action of a formative substance. A substance may determine a certain differentiation or histogenesis, but how it can be "formative" to the extent of determining the entire spatial pattern of an embryo has never been made clear. According to the evidence at hand, organizers and reorganizers of early embryonic and reconstitutional stages represent relatively high gradient-levels, though there is disagreement on this point as regards the so-called "primary organizer" in amphibians (pp. 123–27). There are, at present, few data concerning presence or absence of gradients in insect eggs and none on *Platycnemis*. However, a polar oxidation-reduction gradient is present in the *Drosophila* egg (Child, 1942, 1943*b*). Very distinct oxidation-reduction gradients have been observed in eggs and embryos of two species of caddis flies (Child, unpublished). In these three cases the anterior region of the egg is the high end of the gradient preceding embryo formation and in early stages. If a gradient is present in the *Platycnemis* egg, the organizer is in its low end. It is at least allowable to suggest that, instead of preventing passage anteriorly of a substance or destroying it, the experimental procedures irritate or stimulate regions anterior to the ligature or the killed region to such a degree that polar gradient pattern is virtually obliterated. According to this suggestion, inhibition of development in this case is brought about in a manner somewhat similar to the decrease in scale of organization of the hydranth primordium

[16] Seidel, 1926, 1928, 1929*a*, *b*, 1935.

and the differential inhibition of planarian head regeneration by the injury and stimulation resulting from the cut at the posterior end of the piece. There is, of course, no nervous system in the insect embryo; but the nucleated cortex can undoubtedly transmit stimuli to some extent over short distances and probably with a decrement in intensity. The presence, migration anteriorly, and organizing action of a "formative substance" in the *Platycnemis* egg remains to be demonstrated.

INDUCTION IN ECHINODERM DEVELOPMENT

Runnström and his co-workers, Hörstadius and Lindahl, have regarded polarity in the sea-urchin egg as consisting of two preformed, specifically different substance gradients with highest concentrations at animal and vegetal poles, respectively, and interpenetrating each other. This concept was first presented in terms of "animal" and "vegetative" substances, but more recently specifically different animal and vegetal metabolisms have been postulated (Child, 1941*a*, pp. 241–46).

It is well-known that in the fourth cleavage of sea-urchin eggs four very small cells, the micromeres, are formed at the vegetal pole. These are regarded by the Runnström group as representing the highest concentration of vegetal substance or the highest level of the vegetal type of metabolism. In an extensive and beautiful series of experiments on isolation, transplantation, and implantation of blastomere groups, single blastomeres, and even single micromeres, Hörstadius has demonstrated that development may be determined as more animal or more vegetal in character, according to position in the polar pattern represented by host blastomere groups and the blastomeres added to them.[17] Here only induction or organizer action by micromeres need be mentioned. One or more of these cells implanted at more animal levels tend to induce development characteristic of levels farther vegetal than would have occurred without the implanted micromere or micromeres. By micromere implantation in the region of the animal pole small entodermal invaginations and even complete reversal of polarity have been induced. This organizing—actually reorganizing—action has been interpreted by Hörstadius as a specific effect resulting from the high concentration of specific vegetal substance or the specific vegetal metabolism of the micromeres.

There is no evidence that the micromeres are organizers in natural development. Moreover, formation and invagination of entoderm may occur in their absence. In starfish development no micromeres are formed; the blastomeres at the vegetal pole are the largest cells of the blastula, but entoderm forms and invaginates as in the sea urchin. Oxidation-reduction indicators show only a very slight gradient of oxidase activity and reduction decreasing from the animal region vegetally in early cleavage stages and blastula of both echinoid and starfish. The sea-urchin micromeres do not differ distinguishably from adjoining cells as regards oxidation-reduction characteristics. Before gastrulation, however, there is a great increase in rate of reduction in the prospective entodermal region and including the micromeres, and the cells formed from the micromeres become the most rapidly reducing cells of the echinoid late blastula (Child,

[17] Hörstadius, 1935, 1936*a*, *b*; and accounts of his work, other work, and conclusions of the Runnerström group by Weiss, 1939, pp. 269–88; Child, 1941*a*, pp. 240–46, 436–46; Needham, 1942, pp. 476–502.

1941*b*, 1944). This high rate of reduction is perhaps associated with the rapid growth and probable protoplasmic synthesis in entoderm and mesenchyme. A reduction gradient extends apically and obliterates the slight original gradient from the animal pole over more than half the embryo. These data suggest that the vegetal gradient of Runnström *et al.* may not be preformed but develops during blastula stages, that the character of metabolism in the vegetal region may become different before gastrulation from that of earlier stages, and that the descendants of the micromeres share in any such change. However, it is not yet determined whether or to what extent specific substances or quantitative metabolic factors are concerned in the inductive and reorganizing action of the micromeres. Perhaps they induce entoderm when implanted because they become the most rapidly reducing cells of the embryo.

The primary polar pattern of the sea-urchin egg is apparently determined in the ovary, the free pole becoming animal, the attached pole vegetal (Child, 1941*a*, pp. 660–62). Growth of the gonad in a direction at right angles to the axis of oöcyte attachment may initiate dorsiventrality. Evidence of a gradient of oxidase activity decreasing from the tip of the gonad subdivisions has been obtained (Child, unpublished). Oöcyte axes are in all possible directions with respect to the axiate pattern of the maternal organism, and the axiate patterns of larval sea-urchin development are very different from the adult pattern.

THE ORGANIZER PROBLEM IN AMPHIBIAN DEVELOPMENT

DESIGNATIONS AND DEFINITIONS

The demonstration of determination or induction by the optic cup of lens formation from overlying ectoderm in amphibians and Spemann's classical experiments on induction of amphibian neural development and of embryos by pieces of the dorsal lip of the blastopore implanted in the blastocoel led him to designate the dorsal lip as the primary organizer of amphibian development. These and other experiments on amphibian development led to a tremendous volume of investigation, largely on amphibians, concerned with various aspects of induction and organizer action, that is, with the action of a part of an embryo in determining developmental changes in another part. Experimentation was soon extended to other vertebrates, and inductors and organizers were discovered in invertebrates, as is evident from preceding sections of this paper. Present-day embryology is in large measure dominated—one is tempted to say "organized"—by the concepts of inductor and organizer, very commonly in the form in which they have developed from experimentation on amphibian development. As so conceived, an inductor in development is something that determines or induces a developmental effect. Numerous completely extraorganismic agents may, of course, be inductors. An organizer not only induces a particular effect of some sort in a region external to itself but must determine or induce a spatial pattern, an organization, in the region affected. In the amphibian experiments implantation in the blastocoel of many tissues, living or dead, from many organisms, of various organic substances in semisolid carriers, and of some inorganic substances has often resulted in development of some degree of neural differentiation in overlying ectoderm. Neural differentiation of ectoderm has also resulted from contact of ectoderm with various tissues or substances in

explants. This has been regarded as induction by these tissues and substances. The neural differentiation in these cases is usually without a definite axiate pattern, such as develops under the influence of the living, so-called "primary organizer" of the amphibian embryo. These differences in effect of tissues and substances regarded as inducing merely some degree of neural differentiation and of the natural living inductor which can determine a definite axiate pattern of neural organization have been designated "evocation" and "individuation."[18] The authors of these terms have defined evocation as induction of an embryonic axis without regional characteristics; individuation as induction of an axis with regional characteristics. It seems pertinent to inquire as to the character of an embryonic axis without regional characteristics. Apparently for the authors, it is a neural tube without regional differentiation. But absence of visible differentiation does not prove absence of regional characteristics. How can such a neural tube be induced in a certain direction without regional characteristics? Would not evocation be more correctly defined as induction of an effect without axiate pattern, e.g., differentiation of neural tissue without evidence of organization, such as occurs with many foreign inductors? In his glossary Needham (1942) offers other definitions of these terms as follows: evocation is "that part of the morphogenetic effect of an organizer which can be referred back to the action of a single chemical substance, the *Evocator*"; individuation is "that part of the morphogenetic effect of the primary organizer which cannot with certainty be referred back to the action of a single chemical substance, the evocator." These definitions are, of course, based on amphibian development and primarily on neural induction: they are certainly not generally applicable until much supporting evidence is available. Moreover, Needham's certainty as regards the single chemical substance in evocation does not appear at present to be justified by the evidence at hand. His definition of individuation in negative terms is certainly new, and it is difficult to believe that it is of any value for any purpose. Embryonic individuation involves a spatial pattern of some sort. If more than one substance is concerned in it, the spatial pattern of the substances, not the substances themselves, constitutes individuation.

Inductors or organizers have been demonstrated or postulated in many other organisms besides amphibians; and the concept of induction has been extended to hormones, to substances of nuclear origin, intermediate between genes and characters of the organism, and even to the genes themselves (Waddington, 1939; Needham, 1942, p. 408).

INDUCTORS OF AMPHIBIAN NEURAL DEVELOPMENT

The experimental attack on the problem of the nature of induction and organizer action in amphibian development has centered about neural induction. The experimental data have been presented, discussed, and analyzed so often and have played so large a part in recent books on embryology that they need not be considered at great length here.[19] At first it was taken for granted that neural induction and organization was a specific function of the living organizer, the dorsal lip of the blastopore,

[18] Needham, Waddington, and Needham, 1934. See also Needham, 1942, p. 125.

[19] See, e.g., Huxley and De Beer, 1934; Weiss, 1935, 1939; Spemann, 1936, 1938; Child, 1941*a*; Needham, 1942.

which gives rise to chorda-mesoderm; but further experiment soon seemed to indicate that this was far from true. Organizer tissue from the dorsal lip was apparently still capable of neural induction after the following treatments: crushing; narcotization, though how long after implantation narcosis continued may be questioned; boiling; freezing; drying; some hours in 20 per cent HCl; several days in ether; extraction by ethyl ether or petroleum ether for several hours; several months in alcohol, followed by impregnation with xylol and paraffin; etc. Other amphibian material—for example, ventral ectoderm, yolk cells, fertilized and ovarian eggs; egg nuclei, without effect when living, brought about neural differentiation after being killed by boiling. It was also discovered that the action was not species-, genus-, order-, or even class-specific. Neural differentiation was obtained with chick primitive streak, fish, and lamprey organizer placed in the amphibian blastocoel. Other living amphibian tissues, limb buds, and the induced developing neural tissue also brought about neural differentiation when implanted. Still further experiment showed that various adult tissues from most animal phyla, either after killing or in some cases while living, were similarly effective in some degree. Neural differentiation was also obtained with neoplastic tissues, many organic substances, including polycyclic hydrocarbons, some carcinogens, certain oils, nucleic acid, some other acids and bases, proteins, including slight effects by egg albumin, and some inorganic substances. Slight induction by certain plant tissues has been reported, but Needham concludes that plant tissues do not induce (p. 175). Induction has been obtained in the presence of cyanide and in the absence of oxygen, but the embryo is not highly cyanide-sensitive or sensitive to low oxygen in earlier stages.

Assuming, for the present, that all these cases of neural differentiation are actually induced by the various tissues and substances, it appears evident from these lines of experiment that the capacity to induce is not restricted to any particular kind of tissue from any particular organism but is of very general occurrence. If the inductor is a single specific substance, it must be very widely distributed in organisms and extremely stable over a wide range of temperature and treatment with chemical agents. Also, it must be freed from some tissues only when they are living; from others, only after they are dead. If different substances from different tissues are inductors, the probability that induction results from a specific action or actions decreases. Some of the various inductors determine extensive inductions; others have little effect; and frequencies of induction vary widely. Results obtained with particular foreign inductors are highly variable. In general, dead inductors appear to be less effective than the living, natural inductor. Invertebrate tissues are apparently less effective than those of vertebrates, and certain glandular vertebrate tissues have been found more effective than other tissues. There is also great variation in character of the inductions produced, even by the same inductor. They may range from thickenings of epidermis, a single lens, balancer, etc., to large complexes, sometimes with several neural tubes, and various other organs. Nose, optic and otic vesicles, balancers, gland tissue, and other parts may develop, in addition to central neural structure or even when it is completely absent. Some of the differences in character of

induced structures are regarded as resulting from differences in age of the host; that is, the system reacting to the inducing action determines the character of the resulting differentiation. In general, implanted pieces of the dorsal blastopore lip usually induce more or less nearly normal neural plates and tubes and other embryonic organs in normal or nearly normal pattern; but effects of foreign inductors are frequently chaotic. In short, there is apparently no definite relation between character of the parts induced and nature and origin of foreign tissue inductors. However, Chuang (1940*a*, *b*) claims to have found characteristic differences in inducing action of amphibian liver and mouse kidney, between the same tissues living and boiled, and with mouse kidney after periods of boiling differing by only a few minutes. He assumes that different specific substances are freed from the different tissues and by the different periods of boiling. Needham (1942, pp. 172 and 279) and Brachet (1945, p. 434) accept this work without criticism and regard it as highly important.[20]

In the amphibian exogastrulae, produced by cultivation of early stages in salt solution, differentiation of organs occurs in the evaginated entomesodermal mass; but the ectoderm connected with this mass by a slender tissue strand shows no differentiation. These cases have been regarded as providing conclusive evidence that the inductor is necessary for neural and other differentiation of ectoderm. However, the possibility that physiological condition of the ectocerm may be altered in the exogastrulae seems to have received little or no attention.

Implantation into the larval eye cavity and into the peritoneal cavity of pieces of prospective epidermis and prospective neural plate from blastulae before action of the organizer resulted in development of neural and other tissues; it has been suggested, however, that these regions of implantation are not indifferent but possess complex organizing capacities. Ectoderm explanted in a weak salt solution (Holtfreter solution), before organizer action, remained alive for long periods but showed no differentiation. If natural organizer was present with the ectoderm, neural tissue and other organs developed. Neural differentiation was obtained even when pieces of prospective epidermis were laid on boiled organizer in Holtfreter solution.

CERTAIN CHEMICAL ASPECTS OF THE INDUCTOR PROBLEM

That induction by the dorsal lip of the blastopore, the so-called "primary organizer," may be due to a chemical substance or substances was apparently first suggested by Spemann and Mangold (1924). Holtfreter and others also postulated a substance or more than one. Attempts to discover an inductor substance and to determine its character led to another line of extensive investigation. If chemical substance is involved in the action of dead foreign inductors, it is apparently not readily dissolved or removed by boiling or by most other treatments to which these inductors have been subjected, but evidently reaches the host readily. Certain investigators reported that glycogen decreased in the dorsal lip more rapidly than elsewhere during invagination and suggested that it might be associated in some way with induction, but with another method no such decrease was found. In some experiments with glycogen, induction was ob-

[20] See the discussion of this work, Child, 1941*a*, pp. 473, 474.

tained; with more complete purification, inducing power decreased or was absent. It was maintained by Heatley, Waddington, and Needham (1937) that some agent associated with glycogen was highly effective. Brachet (1939) claimed that induction could occur in the presence of agents inhibiting glycolysis and suggested that proteins may be important factors in induction. At present the evidence indicates that neither glycogen nor a metabolic product of it is essentially concerned in induction (e.g., Jaeger, 1945). As Weiss (1935, p. 664) has noted, however, the glycogen hypothesis is important because it suggests that metabolic activity is involved—at least in natural induction.

Extensive investigations by Needham, Waddington, and co-workers led them to the conclusion that the natural inductor (evocator) is a definite chemical substance, probably a steroid, or sterol-like in nature. According to this view, the inductor substance is present in inactive or masked form in the whole embryo but is liberated in only the dorsal lip region during gastrulation. It is probably widely distributed elsewhere, and treatment of foreign tissues by boiling and otherwise may serve to unmask or liberate it. Also, apparent induction by certain substances may consist in liberating the inductor substance from host cells. In Needham's recent book this hypothesis is presented at length and is held to be the most satisfactory one (Needham, 1942, particularly pp. 165–216). Protein residues of mammalian brain were found more effective as inductors than lipid extracts by Barth (1939*a*); this led him to suggest, as Brachet did about the same time, that the natural inductors are proteins. More recently Brachet (1945, pp. 427–43) has discussed at some length the probable or possible significance of proteins, nucleoproteins, and nucleic acid in induction.

It is a point of considerable interest that substances in liquid form injected into the blastocoel apparently do not usually induce, even though they may induce when implanted after mixture with egg albumin and coagulation, or mixture or emulsification with some other carrier to make a semisolid mass. The semisolid condition perhaps aids in localization of effect, but it may also produce irritation. It has been suggested repeatedly that at least some of the tissues and chemical substances which apparently act as inductors do so indirectly by liberating the inductor from amphibian cells. This suggestion was made by Barth (1937) and by Barth and Graff (1938) as regards cephalin and other toxic substances that produce cytolysis in the blastocoel, and by Okada (1938) concerning induction by fuller's earth, silica, and calcium carbonate. The same suggestion was advanced by members of the Cambridge group. Induction by microcautery in the blastocoel has been reported by Cohen (1938). More recently, extensive experiment with particular attention to occurrence of cytolysis has led Holtfreter (1945) to the conclusion that "in most, if not all, cases reported in the literature where chemical substances, dead, or foreign tissues served as 'inductors' their effect may be regarded as an indirect one mediated by cytolyzed cells of the host."

The effect of methylene blue is of particular interest in this connection. A piece of living ventral ectoderm does not naturally induce; but after cultivation for a day or two in Holtfreter solution containing methylene blue, it not only becomes a relatively effective inductor but may itself undergo differentiation

into neural tissue.[21] In this case the effective agent, the dye, is a liquid, not a semisolid. It was thought at first that these results might be due to the metabolic acceleration by the dye, since increase of respiration by methylene blue has been demonstrated in certain cases. However, Needham argues that, since the increase of oxygen uptake in the ventral ectoderm was found to be small (45 per cent), methylene blue acts by releasing the evocator, and that "in all probability" neutral red and Janus green, which are not known to increase respiration, may "activate the primary evocator." It is perhaps open to question whether an increase in oxygen uptake of 45 per cent is "relatively small." From some viewpoints it might be regarded as large. It may also be noted that neutral red is also highly stimulating to *Paramecium* and other ciliate protozoans and to flatworms and various other forms. Even in concentrations of 1/50,000 and lower, locomotion of the ciliates becomes much more rapid; and though the animals become stained at first by the oxidized dye, ectoplasmic reduction may begin in a few minutes without external decrease of oxygen. Janus green is also stimulating, but in much lower concentrations; and, although the animals do not reduce it, even to the red form, without some oxygen decrease, their reducing power is apparently increased—at least for a time, until the reducing system is injured by toxic effect (Child, 1945*a*). Needham's conclusion that these dyes induce by activating the primary evocator does not appear, at present, to follow necessarily from the evidence. Moreover, experiments with methylene blue have led Holtfreter (1945) to include it among the agents which produce inductive effects by cytolysis of both inductor cells and of host cells. Perhaps it activates or liberates the evocator from the host cells. However, extremely high concentrations are used in some of Holtfreter's experiments. If methylene blue acts in this way, neutral red and Janus green probably also act indirectly by cytolysis of cells. The concentrations of these dyes used by Beatty, De Jong, and Zielinski (1939) are highly toxic to many organisms. The possibility remains that cytolysis may liberate merely a nonspecific stimulating agent. It is perhaps of interest in this connection that in *Paramecium* and other ciliates intracellular reduction of methylene blue, some other dyes, and indophenol usually occurs rapidly without external oxygen decrease as cytolysis approaches or begins, unless concentrations are high.

Weiss (1935) holds that available evidence favors the view that the relation between "dead inductors and their products is entirely unspecific." In other words, these inductors merely activate. It was also suggested by Barth and Graff (1938, p. 386) that neural induction may be "a reaction to a stimulus, much as an egg reacts to parthenogenetic agents." Many toxic agents, if applied for a short time, activate eggs. Also, it is generally recognized that many agents which are toxic and inhibitory in higher concentrations stimulate in low concentrations. Barth considers it unnecessary to assume with Waddington, Needham, Nowinski, *et al.* (1936) that various substances, methylene blue among them, induce by releasing the evocator or inductor substance. He suggests that their inductive action may be direct, substituting for the stimulus by the natural

[21] Waddington, Needham, and Brachet, 1936; Waddington, Needham, Nowinski, *et al.*, 1936; Beatty, De Jong, and Zielinski, 1939; Needham, 1942, p. 189.

inductor. He points out further that dead cells may produce various toxic substances which may stimulate living cells and that there is no conclusive evidence that the dead cells contain the natural inductor or evocator.

The chief results of the great volume of investigation concerned with amphibian inductors and induction appear to be two: first, occurrence of neural induction, not only in the presence of the dorsal blastopore lip or parts of it but also in the presence of many tissues of widely different origin and character, living or dead and treated in various ways, many organic chemical substances of various constitution, certain dyes, some inert inorganic substances, and microcautery; and, second, the setting clearly before us of the problem of the nature and manner of action of the inducing factor or factors. This problem has by no means been solved. It has not yet been proved that the evocator is a steroid substance, that there is a single widely distributed evocator or inductor in inactive or masked form which is liberated by various apparently inducing agents, or that neural induction is a specific action. The experimental evidence is open to other interpretations. At present it appears entirely possible that induction by foreign living and dead tissue inductors and chemical substances may be primarily nothing more than an activation, either by products of cytolysis of host cells, as Holtfreter maintains, or by the agents themselves. And, finally, even if future work demonstrates that differentiation of neural tissue may be induced by a particular chemical substance of very wide distribution in organisms, we know no more than before concerning the organization of the nervous system and the embryo in natural development.

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INDUCTION AND ORGANIZATION

At present there seems to be fairly general agreement that with dead and foreign inductors the character of the induced effect, that is, the morphological result, is, at least in large part, determined by the condition of the reacting system, the ectoderm. An "individuation field" has been postulated as determining the regional character of parts developing in the presence of dead and foreign tissues and chemical substances, that is, whether they resemble cephalic or postcephalic regions (Waddington and Schmidt, 1933; Waddington, 1934). Spemann (1938, p. 369) pointed out that, with the progress of experiment, increasing importance has been assigned to the reacting ectodermal system in determining the character of structures induced by dead and foreign inductors. It was also noted by Weiss (1935, p. 666) that less effective agents presumably require more time than stronger; consequently, the capacity of the reacting ectodermal system to give rise to particular differentiations may differ with inductors of different intensity when the threshold of induction is attained. There is also another possibility which apparently has received little attention, that is, that differences in degree or intensity of action—purely quantitative differences—may themselves play a part in determining character of the effect. Quantitative differences in the external medium are certainly important factors in determining the character of development, even to presence or absence of parts. Is it not to be expected that they may play a part in determining the effect both with dead and foreign tissues and chemical substances and with the living, natural inductor?

Even if the various tissues and sub-

stances regarded as inductors give no information concerning the problem of organization, there is still the possibility that the natural neural inductor—the dorsal lip of the blastopore and regions anterior and lateral to the lip at the beginning of gastrulation—may throw some light on this problem. This region was called the "primary organizer" by Spemann, and Needham and many others have accepted this designation. Surely the primary organizer of the amphibian embryo may be expected to illuminate the problem of organization.

Pieces of this organizer region above a certain size, implanted in various locations, not only induce neural differentiation in ectodermal cells which would otherwise form epidermis but usually determine development of orderly character, e.g., more or less nearly normal neural plates or parts of plates which become neural tubes and differentiate into nervous systems or parts of systems, distinguishable as brain or more posterior parts. Other embryonic organs may also develop in more or less orderly and normal pattern, and a complete embryo often results. This embryo may develop from a region which includes none of the prospective neural tissue and with its longitudinal axis in a direction different from that of natural development, though there appears to be a factor which is, to some degree, effective in determining that longitudinal axes of the natural and the induced embryo usually approach the same direction. These inductions differ from those appearing with dead and foreign inductors in that they constitute a definite pattern of organization approaching or attaining that of natural development.

Spemann distinguished different regions of the primary organizer as head, trunk, and tail organizer. Experiment has shown that there is a gradient of inductive capacity decreasing anteriorly and also laterally in the dorsal inductor region preceding invagination. The region of greatest inductive capacity, invaginating first and moving anteriorly beneath the prospective neural ectoderm, becomes the head organizer. Parts anterior to it before invagination, invaginating later, and becoming posterior to it after invagination, are successively trunk and tail organizers. However, it was found that this apparent specificity of organizing action is by no means absolute. For example, a head organizer implanted at trunk-level or even at tail-level may induce a complete embryo, or a partial embryo defective anteriorly. A trunk or tail organizer at head-level may induce a complete embryo and at tail-level a partial embryo, defective anteriorly. The head organizer appears in general to be more effective than other organizer levels. These results were interpreted in terms of the predominance of one or the other of the two systems concerned—the organizer system and the reacting ectodermal system. If it is true that absence of complete dominance of organizer levels in determining regional levels of the induced structures results from predominance of differences in reaction at different levels of the ectodermal system over the differences at different levels of the organizer, conclusions and questions of considerable importance follow. According to this interpretation, the organizer induces differentiation, but the reacting prospective neural ectoderm may determine the body-level or levels which that differentiation represents. No definite boundary between head, trunk, and tail organizer can be distinguished; nor are there any distinguishable boundaries in the ectoderm which might be correlated with the

cases in which it supposedly predominates in determining body-level of the induced differentiation. As already noted, there is a gradient of inductive capacity in the organizer; and head, trunk, and tail organizers lie at different levels of that gradient. If the reacting ectoderm can also determine body-level of the induced structure, it seems necessary also to postulate a gradient of reactive capacity, decreasing posteriorly, in the prospective neural ectoderm. Moreover, the gradients of organizer and of ectoderm must be similar—at least in certain respects—if both may play a part in neural differentiation and either may be predominant in determining body-level. After invagination one of these two gradient patterns underlies the other, and both decrease in effectiveness in the same direction. Spemann postulated a "Bahnung," a predisposition toward neural differentiation in the prospective neural ectoderm. This, if actually present, appears to be a real axiate pattern, similar, as regards regional neural differentiation, to that of the organizer. However, presence of a predisposition toward neural differentiation and toward regional differences in such differentiation in the prospective neural ectoderm was not confirmed in further experiments by Holtfreter (1933*a*, *b*). According to Holtfreter, the host organizer determines the regional characteristics of the induced structures.

In either case certain questions arise. If a regional pattern is present in the organizer only, it is evidently organized. If a regional pattern is also present in the prospective neural ectoderm, it also is organized. What is the nature of the regional pattern wherever it is present, and how and when did it originate? We cannot hope to solve the problems of induction and action of the so-called primary organizer until we find an answer to such questions.

The questions whether specific or qualitative differences are or are not present at different levels of the natural inductor and to what extent head, trunk, and tail organizers represent these differences have been discussed from various angles. Extensive experiment has led Dalcq (1940) to the conclusion that results obtained with different parts of the living organizer can be interpreted in terms of quantitative regional differences and that postulation of specific regional differences is therefore unnecessary. If regional differences in the organizer are quantitative, regional differences, if present in the prospective neural ectoderm, are probably also quantitative. However, a series of inducing substances with specific effects has been postulated by Holtfreter (1934*a*, *b*, and other papers). Regional differences in susceptibility to lithium in the organizer at different developmental stages led Lehmann (1938 and earlier papers) to conclude that regional differences specific for stage of development are present at different levels; but his evidence for specificity does not appear conclusive, in view of the fact that use of wide ranges of concentration and of different agents often indicates that susceptibilities of early developmental stages which may appear to be specific, with less extensive experiment, are not really so.

The question of the existence of specific substances at different organizer levels was discussed by Waddington (1938) with the conclusion that they are present. He called them "eidogens," form-producers—again the same old concept of formative substances under another name. Needham (1942, pp. 172 and 279) speaks of morphogenetic hormones and holds that the evidence in-

dicates the presence of different specific substances at different levels of the primary organizer. His definition of individuation (p. 113) indicates his position. In this connection he refers again to the work of Chuang, which was mentioned above (p. 115) and which he regards as in line with his view.

A substance can undoubtedly determine a definite histogenesis. But how can it determine morphogenesis which involves a definite spatial pattern with regional differences in growth rate and character of differentiation? The differences at different levels of the central nervous system appear to be primarily differences in spatial pattern rather than qualitative chemical differences, such as might result from regional specific differences in the organizer. Other specifically differentiated organs develop at different body-levels, but their specificity becomes evident only gradually in many organisms; in early stages of development there is no indication of it. Needham's position as regards specificity in induction is rather strikingly shown in his assertion that a tendency, shown by Spemann and others, "to abandon too readily the search for logical consistency in the chemical facts" and to regard all induction effects as unspecific is "a counsel of despair" (Needham, 1942, p. 186). To some biologists, who see in the development of many organisms a progress from nonspecific or less specific to increasingly specific, attempts to interpret experimental evidence in terms of nonspecific, quantitative patterns and relations in early development appear neither illogical nor a counsel of despair. To them evidence indicating absence of specificity in early stages suggests, rather, that they may be approaching the beginnings of developmental pattern. Moreover, they are often inclined to believe that the burden of proof is on the seeker for specificities.

The hypotheses of specificity of induction and of specifically different substances at different levels of the primary organizer pose more problems than they endeavor to solve. Needham himself calls attention to the presence of supposed inductor substance in organisms in which it apparently performs no inductive function, though, of course, it may have other functions. If Holtfreter is correct as regards induction by cytolyzed amphibian cells, there is no widely distributed specific inductor substance. If there are specifically different substances at different levels of the primary organizer which determine regional differences in the induced neural structures, there must also be somewhat similar specificities at different levels of the reacting ectoderm, if it, as well as the organizer, plays a part in determining body-level of the induced structures. In other words, according to this view, both the organizer and the reacting ectoderm are specifically organized before induction. But whether specific regional differences are or are not present, and even if the prospective neural ectoderm has nothing to do with determination of regional differences in induced neural structures, it is evident that the neural inductor, the dorsal lip of the blastopore, called the primary organizer by Spemann, Needham, and others, is not the primary organizer at all. Something must have organized it. The role of primary organizer can be assigned to the dorsal lip only if we ignore more fundamental features and problems of amphibian development. Whatever its character, the organization present must have been determined in some way preceding gastrula stages. We must go back through earlier

stages of development in the search for primary organizing factors.

According to Needham (1942, p. 103), Spemann's conception of the organizer set dependent differentiation at the heart of development; that is, formation of the embryo, is dependent on the organizer. But on what is the organizer dependent? What makes it an organizer, and how did it attain that condition? Needham's acceptance of the organizer as if it were ready-made seems almost to require something more or less similar to Driesch's entelechy, which he says, quite correctly, is now liquidated (Needham, 1942, p. 119). He regards the primary organizer as absolutely necessary for neural development (1939; 1942, p. 156). But if it is true that the prospective neural ectoderm possesses an organization somewhat similar to that of the organizer, we may expect that if we can subject the ectoderm to the proper conditions it will be capable of some degree of neural development quite independently of the organizer.

It was found by Barth (1939*b*, *c*, 1941) that explants, not only of prospective neural ectoderm but of ventral ectoderm, that is, prospective epidermis, of *Amblystoma punctatum*, can develop neural structures from their more anterior regions, that is, from regions about or near the anterior pole, in complete absence of the organizer. He concludes that there is a gradient of capacity for neural differentiation, decreasing from the anterior pole. Confirmation of the occurrence of neural differentiation without organizer has been provided by Holtfreter (1944) for *Amblystoma punctatum* and later (1945) for *Triturus torosus* under somewhat different experimental conditions as regards character of external medium. Holtfreter suggests that the embryonic tissue of *Amblystoma* is more susceptible to dispersive agents than that of *Triturus*. It is perhaps a point of some interest that Barth found the neural differentiation occurring at the more anterior ectodermal levels. As will appear below, this is the "high" region of an ectodermal gradient from early stages on. However, Holtfreter notes no such regional difference in capacity. It seems possible that Barth's experimental procedure may have been better adapted to show such a regional differential if it is present.

The experiment of holding the frog's egg in inverted position following fertilization and in early cleavage, originally performed by Schultze (1894), often resulted in development of twin embryos. More recent experiment along this line has given results of much interest.[22] In these inverted eggs the heavier yolk tends to fall toward the lower, originally anterior pole; but portions of it may fail to reach the lower pole and may remain in various positions. Regions of contact of pigmented cytoplasm with yolk masses tend to develop blastopores, and invagination and embryo development follow. More than one blastopore and organizer may form in a single egg; and there is apparently no region of the egg surface or cortex which cannot give rise to an organizer, a blastopore, and an axiate embryo. Evidently, the region of organizer and blastopore is not definitively and irreversibly determined in the fertilized egg. However, it appears from the latest experimental data on inverted and partially inverted eggs that localizations and orientations of the organizers and blastopores which develop show certain relations to the original gray crescent which becomes the organizer re-

[22] For discussion of these investigations with references to literature see Schleip, 1929, pp. 584–90, 696–715; Child, 1941*a*, pp. 428–30; Needham, 1942, pp. 218–20.

gion; that is, the original pattern is not entirely obliterated.[23] The region of the gray crescent is apparently the "high" region of a gradient, and the experimental evidence indicates that the regional differences in this gradient are quantitative rather than specific, or at least that specific differences are so slight in early developmental stages that, although the egg is evidently organized, the developmental fate of no region is definitively determined.

THE AMPHIBIAN EGG AND EARLY DEVELOPMENTAL STAGES

It was pointed out above that, since both the so-called "primary organizer" and the ectoderm which it is supposed to organize are already organized to some degree, it becomes necessary to look elsewhere for the primary organizer. Two visible substance gradients—the yolk gradient and the pigment gradient—allow little room for doubt that a metabolic gradient is, or has been, present during the ovarian development of the egg. The yolk spherules increase in size and number from the anterior pole throughout the egg (Bragg, 1939), and the relative volume of cytoplasm decreases in the same direction. Since yolk is a metabolite, it is difficult to believe that the very distinct yolk gradient originated without a metabolic gradient. The other substance gradient is the pigment pattern. The amount of pigment differs in different amphibian species; in some it is restricted to the cortex, in others it is also present internally. The pigment, a melanin, is also a metabolite; its distribution in a gradient pattern certainly indicates presence of a metabolic gradient in the anteroposterior axis of the egg. Yolk and pigment gradient appear to be expressions of the same metabolic gradient.

The gray crescent, which becomes distinctly visible after fertilization in some amphibian eggs and is less clearly distinguishable in others, indicates the dorsal side and becomes the region of the primary organizer. Its position is now generally held not to be determined in relation to point of entrance of the spermatozoon; it is said to be distinguishable in some eggs before fertilization (Schleip, 1929, pp. 558–66). That it represents the "high" region of a gradient system of some sort is indicated by the relations to it of the organizers and blastopores which form in inverted and partially inverted eggs.

It appears, then, that the unfertilized ovarian egg possesses, or has possessed, a polar metabolic gradient and a dorsiventral gradient pattern apparently also involving metabolic factors. Except possibly for the region of the gray crescent, there is no evidence that these gradients are not primarily quantitative; and the experiments on inverted eggs indicate that the gray crescent is not specifically different from other regions to such an extent that they cannot be substituted for it. In short, the egg is organized before fertilization, and we have not yet reached the real primary organizer.[24]

[23] Dalcq, 1938; Dalcq and Pasteels, 1938; Pasteels, 1938*a*, *b*, 1939, 1940.

[24] The presence of polar and dorsiventral gradients is recognized by Needham (1942, p. 217), though he apparently does not regard them as metabolic in character. The yolk gradient, he says, probably results from position of the egg in the ovary, with entrance of nutrition from a capillary touching the egg at one point. Actually, however, the circulatory pattern in relation to the egg is very different from that. The other gradient, determining dorsiventrality, he apparently regards as paracrystalline in character and determined by the maternal organism (p. 218). It is far from clear how a paracrystalline pattern can determine development of organizers and blastopores at points where cytoplasm comes into close contact with a yolk mass in the inverted eggs.

It was found by Bellamy (1919) and later confirmed by him (1921) that in 75–80 per cent of full-grown ovarian eggs of *Rana pipiens* the pedicle which attaches the theca surrounding the egg to the ovary is at or within 20° of the equator of the egg. He pointed out that, since a band 40° wide over the equatorial region represents only about 34 per cent of the total egg surface, the relation of egg and pedicle is not a random one. From the pedicle, a system of blood vessels develops over the whole egg surface in the theca, not in the egg itself. On the basis of observations on injected and living eggs Bellamy stated in the earlier paper (1919) that "in every case observed the greater part of the arterial blood supply was restricted to the pigmented hemisphere" and that the blood supply of the unpigmented hemisphere was largely venous. Figure 13, a reproduction of his Figure 3, shows a case in which arterial and venous circulations correspond very closely in distribution with pigmented and unpigmented hemispheres. With further investigation greater variation in orientation of the egg in relation to the pedicle was found, the arterial circulation being largely over the unpigmented hemisphere in some cases. Consequently, the general statement quoted above, "as it first appeared," was withdrawn (Bellamy, 1921); but it was confirmed that in 75–80 per cent of the eggs the pedicle was at or within 20° of the equator. Figure 13 shows the relation in a large number of eggs. The present writer was in close touch with this work and saw the injected and living eggs; in spite of the exceptions, the high degree of uniformity was striking.

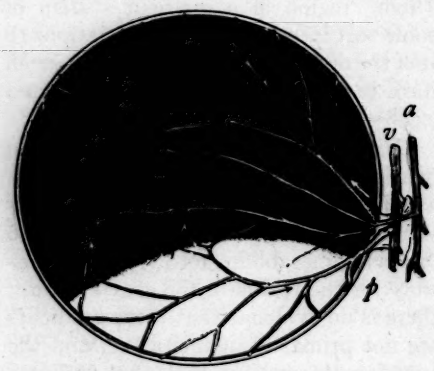

FIG. 13.—Full-grown ovarian oöcyte of frog (*Rana pipiens*), showing the usual relation between arterial and venous circulations in the theca and pigmented and unpigmented regions; *a*, artery, *v*, vein, *p*, pedicle (from Child, 1941*a*, and Bellamy, 1919).

It has seemed necessary to call attention again to these points because it has been repeatedly stated that Bellamy withdrew his earlier statement that polarity might be determined in relation to the circulatory distribution. What he actually did was to point out that the earlier statements were too general. Also, he emphasized the point that the modification of earlier statements concerned only the full-grown or nearly full-grown egg. Unfortunately, he neglected to call attention to the fact that the full-grown egg is not attached to the theca in any way and is soon to escape from it. With the contractile activity of the ovarian egg-strings, change of position of the egg in the theca probably occurs not infrequently. Bellamy was unable to continue the investigation with earlier stages of ovarian oöcytes; but up to the present it appears highly probable that polarity of the frog's egg is determined in relation to the distribution of circulation and that it is a metabolic, as well as a yolk and pigment, gradient. Moreover, it appears possible that dorsiventrality may also be determined in this way, the side on which the arterial circulation enters the theca, and where the oxygen

content of the blood is highest, becoming dorsal. At present this hypothesis of determination of the primary gradient pattern of the amphibian egg is supported by more definite evidence than any other and provides a basis for the yolk and pigment gradients and the indicator pattern of early developmental stages (p. 128).[25]

The point to be emphasized here is this: if the thecal circulatory pattern does determine polarity and perhaps dorsiventrality in the frog's egg, it, not the dorsal lip of the blastopore, is the primary organizer; and if other factors in the ovarian environment of the oöcyte determine its axiate gradient pattern, they constitute the primary organizer. The so-called "primary organizer" of the gastrula is not the beginning but only a step in the progressive organization of the amphibian embryo and of vertebrate embryos in general. In natural development it does not even determine the longitudinal axis of the embryo; that and dorsiventrality are determined much earlier. The real primary organizer, according to this view, is external to the egg but has no direct relation to the axiate pattern of the maternal organism.

Since the yolk gradient and the dorsiventral gradient apparently do not constitute organization for Needham, the question of the origin of the primary organizer of the gastrula with its postulated specific regional characteristics from unorganized earlier stages remains unanswered. Similarly, the origin of the predisposition to regional neural development, that is, the organization of the prospective neural ectoderm, remains completely obscure. To assume that these are in some way products of a paracrystalline state, as Needham apparently does, only raises new questions and answers none. If the paracrystalline state does not constitute organization, we still have to discover how organization originates; if it does constitute organization, then the dorsal lip of the blastopore is still not the primary organizer.

It remains to consider what the early stages of amphibian development actually show as regards pattern. Using a nitroprusside reaction, considered to be an indicator of presence and distribution of sulphydryl complexes, Brachet (1938) has found that what he believes to be sulphydryl proteins occur in a region about the anterior (animal) pole in unfertilized and recently fertilized eggs. Some two hours later the reaction in forms with distinguishable dorsiventrality appears in a region corresponding to the gray crescent of the frog's egg. In later cleavage and blastula stages it appears in the anterior half, sometimes extending farther posteriorly on the dorsal than on the ventral side; and usually the reaction becomes more intense in the dorsal blastopore lip. Brachet regards the reaction as indicating distribution of sulphydryl proteins. Sulphydryl compounds have been regarded as important factors in metabolism. Somewhat later it was reported that various reducing agents induce neural differentiation in explants of ventral ectoderm and that oxidizing agents inhibit such differentiation more or less completely in dorsal explants, consisting of prospective chorda and neural ecto-

[25] Incidentally, it may be noted that the reference to Bellamy's observations by Needham (1942, p. 217) is entirely incorrect. He speaks of Bellamy's "proof" that the egg receives its reserve material from a capillary reaching only one point of its surface, and of the later withdrawal by Bellamy. Actually, there was no such proof. Moreover, the circulation does not reach the egg at only one point but surrounds it, and Bellamy's withdrawal did not concern this point. Such lack of care in reporting the work of others seems inexcusable.

derm (Brachet and Rapkine, 1939). The authors regard these results as in accordance with the data on sulphydryl proteins. According to Holtfreter (1945), both reducing and oxidizing agents induce indirectly by bringing about cytolysis.

Determinations of respiration on pieces from different regions of embryos are not in agreement. According to Brachet (1934*a*, *b*, *c*), destruction of the dorsal lip decreases oxygen uptake in the remainder of the embryo more than does equivalent destruction of an indifferent region. Carbon dioxide production was decreased more by removal of the dorsal lip than by removal of an equivalent region from the ventral lip. Later determinations by Waddington, Needham, and Brachet (1936) on isolated pieces taken directly above the dorsal lip and pieces of ventral ectoderm of *Triton cristatus* showed oxygen uptake of the same order in both. Still other determinations by Brachet (1936), with determinations of total nitrogen, showed CO_2 production and respiratory quotient higher in the dorsal lip than in ventral ectoderm. Oxygen uptake of single embryos was determined by Brachet and Shapiro (1937) by bringing an embryo of *Rana sylvatica* into a tube with lumen slightly less than the diameter of the embryo, with control of its position, and by connecting each end of the tube with a respiratory manometer. Embryos placed with dorsal hemisphere toward one, ventral hemisphere toward the other, end of the tube showed oxygen uptake 47 per cent greater on the dorsal side. In a position 90° from this the two sides showed no difference. According to another series of determinations, by Fischer and Hartwig (1938), rate of oxygen uptake of the anterior half of the blastula is 3.1 times that of the posterior half, that of the dorsal inductor region twice, that of the neural plate 2.61 times that of the whole embryo, and that of the anterior region of the later embryo higher than that of the posterior part. In a still later paper Brachet (1939) finds little difference in oxygen uptake of dorsal and ventral sides in *R. fusca* at the beginning of gastrulation and agrees with Boell, Needham, *et al.* that in the earlier determinations on *R. sylvatica* the time required was so long that invagination was advanced before completion of the determinations, and that the resulting presence of two cell layers on the dorsal side and only one on the ventral side might account for the higher oxygen uptake of the dorsal hemisphere. A question arises as regards this point. Unless there is considerable increase in number of cells and volume of cytoplasm during invagination, the same cells are present on the dorsal side before and after invagination. Is a greater oxygen uptake to be expected after invagination than before?

Respiratory determinations by Boell, Needham, *et al.*, using the Cartesian diver as ultramicrorespirometer, with very small isolated pieces of tissue, are not in complete agreement.[26] For example, in *Discoglossus pictus* and *Amblystoma mexicanum* oxygen uptake of dorsal lip and ventral ectoderm was approximately the same (Boell and Needham, 1939); but in another series of determinations, by Boell, Koch, and Needham (1939), the dorsal lip was 26 per cent higher in *A. mexicanum*. Needham's general conclusion is that oxygen uptake in the dorsal lip is of the same order as in ventral ectoderm. A decrease in respiration from the anterior pole on the dorsal

[26] Needham and Boell, 1938; Boell, 1938; Boell, Needham, and Rogers, 1939; Boell and Needham, 1939; Boell, Koch, and Needham, 1939; Needham, Rogers, and Shen, 1939.

side and a steeper decrease on the ventral side was found by Boell and Nicholas (1940; also Boell, 1942),[27] but they maintain that after correction for regional differences in yolk content actual quantitative differences in respiration do not appear. However, it is maintained by Barth (1942) on the basis of determinations on explants from *A. punctatum*, *A. opacum*, and *Rana pipiens* that, even after correction for differences in yolk content of cells, respiration decreases from the anterior pole posteriorly on the ventral side, while on the dorsal side the dorsal lip respires at about the same rate as the anterior pole, with a sudden drop in the posterior, vegetal region. Barth, quite correctly, points out that the determinations by Boell, Needham, *et al.* are meaningless because differences in yolk content between dorsal lip region and ventral ectoderm were not taken into account. The dorsal lip cells contain more yolk than those of ventral ectoderm. If they respire at the same rate without correction for yolk, the metabolizing protoplasm of the dorsal lip must actually respire at a considerably higher rate than that of the ventral ectoderm.

The lack of agreement in the various determinations is evident and appears to illustrate some of the difficulties and uncertainties of respiratory determinations on small isolated pieces. It does not appear that there is any particular advantage in the use of the very small single pieces with the Cartesian diver. The effects of isolation on these pieces and of possible cytolysis occurring during the determinations are not known. Would not use of several pieces from the same region of different individuals and of larger size be less likely to introduce complicating factors and avoid individual variations? The extreme sensitivity of the Cartesian diver method does not seem to present any particular advantage in these determinations. Most of the determinations are without definite significance because of failure to take into account differences in yolk content of different regions, as Barth has noted. However, Boell and Nicholas do not agree with Barth as regards the regional pattern of oxygen uptake after correction for yolk by different methods. So far as determinations have been made, it does appear to be agreed that CO_2 production, respiratory quotient, and anaerobic glycolysis are higher in the dorsal lip than elsewhere; and certain histochemical methods indicate more rapid decrease of glycogen there than elsewhere.

[27] In his paper of 1942 Boell stated that the full report of these determinations would appear soon. If it has appeared, it has escaped the writer's notice.

OXIDATION-REDUCTION INDICATOR PATTERNS IN AMPHIBIAN DEVELOPMENT

Reduction of oxidation-reduction vital dyes has been followed in the unpigmented embryos of the European *Triton cristatus* and in the American *Triturus torosus* and *T. rivularis*. In *Triturus*, pigmentation of some lots of eggs is so slight that reduction of dyes can be observed, and a few albino embryos of *T. rivularis* have been obtained.

In neurulae of *Triton*, stained by oxidation of reduced brilliant cresyl blue, reduction under anaerobic condition was found most rapid in the floor of the open neural plate (Fischer and Hartwig, 1936). Anaerobic reduction of the same dye was most rapid in the anterior (animal) hemisphere of *Triton* blastulae and decreased from the equatorial region posteriorly. Gastrulae which gave definite results (60 per cent of the total number) showed a similar pattern, except for more rapid reduction in the

dorsal blastopore lip than on the ventral side (Piepho, 1938).

The regional patterns shown by oxidation-reduction indicators become directly visible in single intact embryos and under properly controlled experimental conditions (e.g., dye concentrations, staining periods, and conditions for reduction) are similar in all individuals of the same stage. While it does not appear necessary to assume that the oxidation-reduction patterns made directly visible by the indicators are always completely identical with regional patterns of oxygen uptake, the indicator methods are unquestionably much more delicate than any present methods of determining regional differences in oxygen uptake and may bring to light features of oxidation-reduction pattern not otherwise shown. Moreover, they are independent of differences in yolk content.

In *Triturus*, these patterns have been followed from early cleavage to tadpole stages (Child, 1943*a*, *c*). Figures 14 and 15 are reproduced here as showing diagrammatically methylene blue reduction patterns of certain stages. Arrows indicate directions of progress of reduction—longer arrows more rapid progress, shorter arrows less. Broken lines in certain figures indicate approximate boundaries of more rapidly reducing regions; that is, they represent regions where rate and progress of reduction decrease rapidly. In the four-cell stage, reduction progresses posteriorly from the anterior pole and more rapidly from a region just posterior to the blastomeres on one side, presumably the dorsal side (Fig. 14, *A*). Figure 14, *B*, shows a more advanced stage of reduction in early cleavage. Here the reduction gradient from the anterior pole and that on the side where separation of blastomeres from the yolk is most rapid, presumably the dorsal side, have extended over the whole region within the broken line. In Figure 14, *C*, an early stage of reduction in the blastula is indicated with anterior and presumably dorsal regions of rapid reduction still distinct; these merge in later reduction stages (Fig. 14, *D*). The reduction pattern of the "primary organizer" of the early gastrula appears in Figure 14, *E*, and that of the late gastrula in Figure 14, *F*. Figure 15, *A*, is the same late gastrula stage in side view; Figure 15, *B*, a still later gastrula with the last not yet invaginated portion of the dorsal inductor and the pattern of the early neural plate. Figure 15, *C* and *D* show the closing blastopore and the developing neural groove with a slight gradient from the blastopore. Stages of neural-plate development with their gradient reduction patterns appear in Figure 15, *B*, *D*, and *E*. In Figure 15, *D*, the slight gradient extending anteriorly from the blastopore is still present; in Figure 15, *E*, it is no longer visible. In the neural plate, reduction decreases posteriorly from the anterior region of the floor and folds. The progress of reduction posteroventrally in regions lateral to the neural plate is indicated in Figure 15, *D*, *E*, and *F*. The gradient pattern of dorsal, lateral, and caudal regions of a later stage is shown in Figure 16. All these embryos were stained within the chorion, after removal of the jelly, by intracellular oxidation of dye (methylene blue in most cases) reduced by sodium hydrosulphite. With reduction by oxygen uptake of embryos in sealed preparations and by sodium hydrosulphite, which is not toxic in the concentrations required, the same patterns appear. In reoxidation, following reduction, the gradient patterns are reversed; that is, regions of most rapid reduction reoxidize the dye least rapidly,

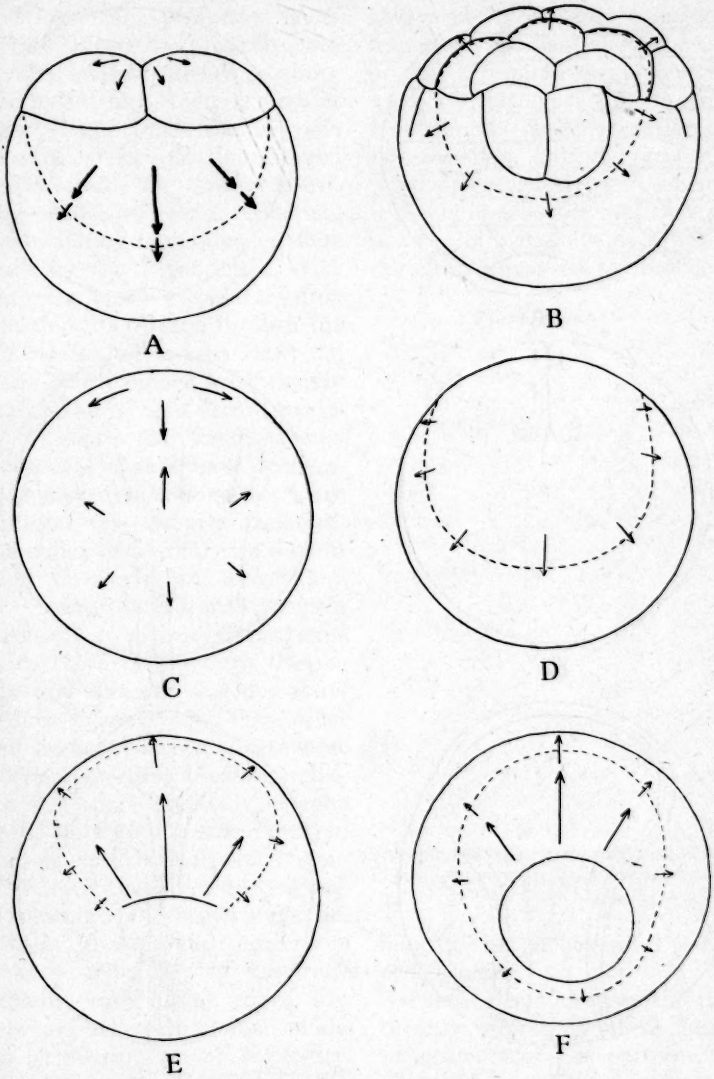

FIG. 14.—Differential reduction in cleavage and blastula stages of the urodele *Triturus*. *A: T. torosus*, four-cell stage with anterior and presumably dorsal regions of more rapid reduction. *B: T. rivularis* albino cleavage, in a more advanced stage of reduction, with anterior and presumably dorsal regions of rapid reduction no longer separate and showing the more rapid progress posteriorly of cleavage on the presumably dorsal side. *C, D: T. rivularis* albino blastula, earlier and later stages of reduction from the presumably dorsal side. *E, F: T. torosus*, early and late gastrula, the dorsal lip reducing more rapidly than any other region. Broken lines in *A, B, D, E, F*, indicate approximately the boundary of the rapidly reducing region where rate of reduction decreases rather sharply (from Child, 1943*a*).

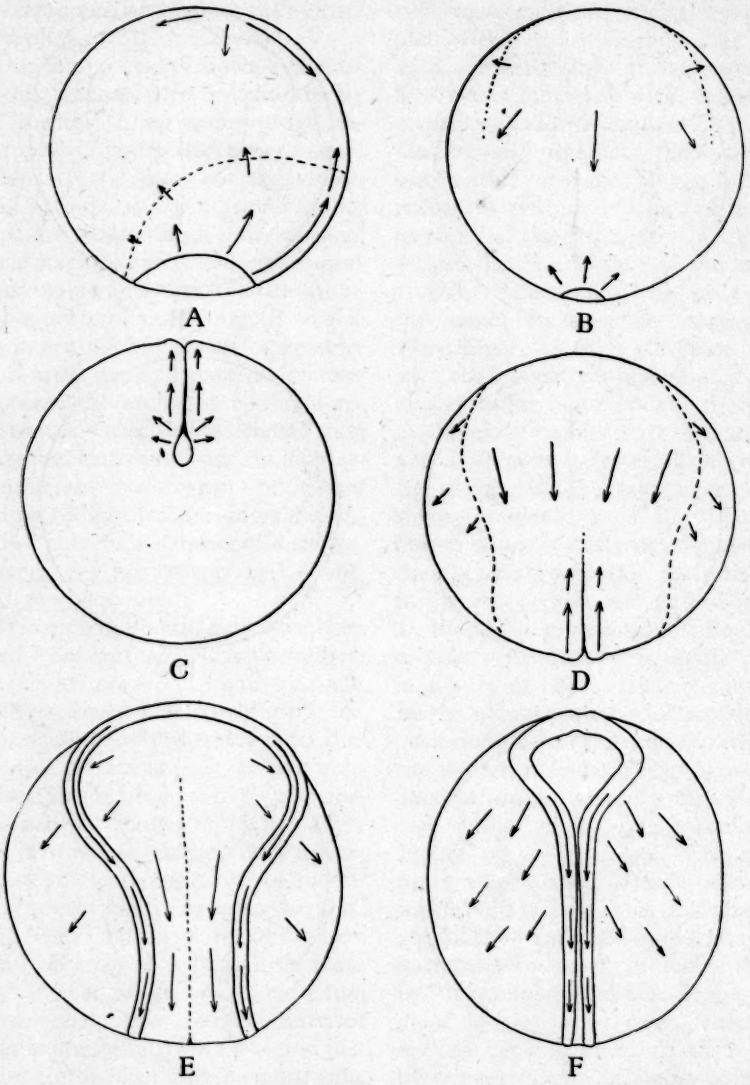

FIG. 15.—Differential reduction in late gastrula and neurula of *Triturus*. *A: T. torosus*, late gastrula from the side. *B: T. rivularis* with most rapid reduction in the faintly indicated anterior part of the neural plate and a small area of relatively rapid reduction still visible anterior to the blastopore. *C: T. torosus*, the developing neural groove with somewhat more rapid reduction progressing anteriorly from the blastopore on each side of it. *D: T. torosus*, early neural plate with reduction progressing posteriorly from its anterior region, anteriorly along the neural groove, and posteroventrally in regions lateral to the neural plate. *E, F: T. torosus*, later neurula stages, showing reduction progressing posteriorly and posterolaterally in floor of neural plate, posteriorly in neural folds, and posteroventrally in lateral regions (from Child, 1943*a*).

regions of least rapid reduction most rapidly. Two factors are probably concerned in this reversal. Following staining by methylene blue, there is more or less differential reduction without oxygen decrease in the external solution. Oxygen concentration inside the chorion is evidently low enough to permit reduction, and the stimulating or catalyzing effect of the dye may also be concerned. With oxygen increase in the external solution after reduction, its passage through the chorion is evidently relatively slow, and the regions which reduce least rapidly obtain enough oxygen to permit oxidation sooner than the more rapidly reducing regions. These data have been reviewed at some length because they leave no doubt that very definite oxidation-reduction indicator patterns are present from early developmental stages and undergo definite changes with progress of development. In general, the patterns agree with those observed by Piepho (1938) in that anterior and dorsal regions reduce more rapidly than other parts; but gradient patterns appear more clearly here, doubtless because reduction occurred more gradually. Piepho brought the embryos into anaerobic conditions all at once. It is a point of considerable interest that the reduction patterns of blastula and gastrula stages are almost identical with the patterns of differential susceptibility in the frog's egg (Bellamy, 1919; Child, 1941*a*, Fig. 53). They are also very similar to the patterns of distribution of sulphydryl proteins reported by Brachet (above, p. 125) and to the considerable number of the determinations of oxygen uptake which indicate presence of a gradient pattern of some sort. However, it was pointed out earlier (Child, 1941*a*, pp. 69–70) that it need not be assumed that an axial dye-reduction gradient must always and necessarily coincide with a respiratory gradient along the same axis or that a respiratory gradient must always be distinguishable in that axis. However, the close association of oxidation-reduction indicator gradients with developmental activity in general makes it highly improbable that they are independent of metabolic gradients. Certain criticisms advanced by Brachet (1945) indicate that he has failed to note these conclusions.

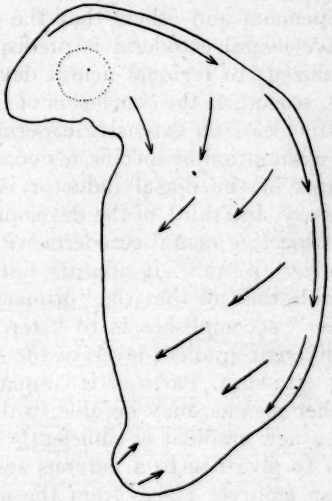

FIG. 16.—Late embryonic stage of *Triturus*, showing reduction progressing posteriorly in dorsal region, posteroventrally in lateral regions, and anteriorly in tail bud (from Child, 1943*a*).

Polarity and dorsiventrality become directly visible in these quantitative oxidation-reduction patterns. There is from early cleavage stages, and probably in the unfertilized egg (lightly pigmented unfertilized eggs have not yet been obtained), a definite organization represented by quantitative gradients. That they are independent of quantitative metabolic factors seems improbable. This organization is present long before

the so-called "primary organizer" comes into action, but it can be altered. The indicator methods give no information as to presence or absence of specific or qualitative regional differences in the gradients. After invagination and movement anteriorly of the dorsal inductor cells the gradient of the inductor underlies the gradient in the prospective neural ectoderm, and both coincide in direction. In the light, first, of the earlier conclusion by Spemann and others that the prospective neural ectoderm is predisposed (organized) to regional neural development, second, of the conclusion of Dalcq (1940), based on extensive experiment, that assumption of specific regional differences in the dorsal inductor is not necessary, and third, of the development of prospective neural ectoderm without organizer (p. 122), it appears not improbable that all that the "primary organizer" accomplishes is to "step up" the different gradient-levels of the overlying ectoderm. Parts of it, implanted in other regions, may be able to determine a new gradient of sufficiently high levels to give rise to a nervous system and an embryo. Pieces from the levels of more rapid reduction of the inductor are more effective in these inductions and reorganizations than pieces from other levels.

Brachet (1939; 1945, p. 404) has reported that in explants of pieces of the neural inductor with prospective neural ectoderm from early gastrulae, subjected to cyanide in salt solution with frequent renewal of cyanide, induction of neural differentiation occurs. Respiration is presumably greatly inhibited under these conditions, and Brachet concludes that maintenance of oxidations at the normal level is not necessary for induction. If induction is effected by a chemical substance produced in the cells of the inductor, it is undoubtedly already present in the early gastrula, and a high level of respiration at that particular time may have nothing or little to do with its inductive action. A more significant experiment would be to determine whether inductive capacity develops in embryos kept in cyanide from early stages. According to Bellamy (1919), neural differentiation can apparently be completely inhibited without complete inhibition of development.

According to this concept of amphibian organization, experimental induction of an embryo or partial embryo by an implanted piece of the dorsal inductor is a reorganization—a reconstitution of an embryonic region which is already a part of a pre-existing organization. In these cases the inductor is actually a reorganizer. Localizations of dorsal lips and blastopores in other regions than the original dorsal side in inverted eggs are also reconstitutions, as are the chaotic organ complexes often developing with dead and foreign tissues and chemical agents.

OTHER AMPHIBIAN INDUCTIONS

As regards induction of lens, otic vesicle, balancer, gills, mouth region, etc., it need only be noted here that the experimental evidence points very clearly to the conclusion that the specificity of reaction, as regards organ or organ system formed, is determined by the reacting system, not by the inductor. Induction of lenses in the absence of optic cups, induction of other organs, even legs, and the regional limitations of these inductions provide still further evidence in support of this conclusion. As differentiation progresses, different regions develop specifically different capacities to react to induction by development of particular organs. There seems to be no

conclusive evidence that the inductors in these cases do anything more than step up the metabolism of the reacting system relatively to other parts, either directly or indirectly through cytolyzed host cells.

INDUCTION AND OXIDATION-REDUCTION PATTERNS IN OTHER VERTEBRATES

In teleosts pieces from the posterior part of the embryonic shield, which represents the dorsal lip of the blastopore, can induce a new embryonic axis when implanted elsewhere. In inductions within the embryonic shield by implants the axis of the induced embryo is usually in the same direction as that of the host embryo. In extraembryonic regions, where there is little or no gradient, it is determined by the inductor. Isolated pieces of the blastula are capable of reconstituting embryonic primordia without inductor action. Luther regards the natural inductor as quantitative in action, that is, as an activator.[28]

The pattern made visible in teleost development by oxidation-reduction indicators is very similar to the amphibian pattern. The dorsal inductor appears as the region of most rapid oxidation and reduction of indicators early in development, in one species in the undivided blastodisk, with a gradient decreasing anteriorly and laterally. This pattern persists through invagination, and the ectoderm overlying the inductor also becomes a region of rapid oxidation and reduction. In one species there is apparently a slight gradient from the apical pole in early stages; in another, presence of such a gradient is doubtful. A gradient decreasing from the anterior end of the embryo becomes clearly distinguishable before the head forms. From this stage on, two opposed gradients are present, as in the amphibian after tail development begins (Child, 1943*d*, 1945*b*). The teleost embryo, like the amphibian, is organized long before the "primary organizer" acts. Moreover, isolated pieces of blastulae can reconstitute embryonic axes. Here again, though some differences in stage of appearance of the two longitudinal gradients occur, differential susceptibility shows a pattern of the same general regional character as the oxidation-reduction indicators (Hyman, 1921).

Development of embryonic axes in birds has been obtained, not only with living, but also with boiled, parts of the primitive streak, and induction is not species-specific. Chick primitive streak implanted in a rabbit embryo *in vitro* may induce neural-plate formation, but rabbit streak in rabbit embryos has given uncertain results (references in Child, 1941*a*, pp. 483–85).

In the chick embryo differential dye reduction shows that the primitive streak and particularly the region of the node are characterized very early by the highest rate of reduction in the embryo (Rulon, 1936). Once more the similarity of the reduction pattern and that of differential susceptibility is called to attention (Hyman, 1927).

The same questions arise concerning these inductors in other vertebrates, as in amphibians. Specificity of their action is not demonstrated, and the fact that it is not is no counsel of despair (p. 121) but merely suggests that early development may not always involve regional specificities to the degree that those who, like Needham, approach problems of development in terms of specific biochemical substances may be inclined to believe.

[28] Oppenheimer, 1936*a*, *b*, *c*; Luther, 1935, 1936*a*, *b*, 1937.

AXIATE PATTERN IN GENERAL

The problem of the nature of the protoplasmic factors determining axiate pattern is so closely associated with the organizer problem that it seems necessary to call attention to experimental data concerned with certain features of axiate pattern in development. And first it must be emphasized that questions of the origin and nature of axiate pattern concern multicellular complexes, isolated pieces capable of reconstitution, whole and partial embryos of various stages, as well as single cells, whether eggs or other reproductive cells.

Graded differentials in oxygen uptake and, in some cases, of CO_2 production, and also in rates of oxidation-reduction of indicators, are characteristic features of axiate pattern in developmental stages, and adults of many organisms.[29] Graded differences in susceptibility or sensitivity to many chemical and physical agents have also been shown to be characteristic features of axiate pattern in many forms. As regards differential susceptibility, the following points are to be noted. First, differential susceptibility of the earlier developmental stages of many organisms and adults of many of the simpler animals, as indicated either by cytolysis or by differential modifications of development, or by both, has been shown to be the same, that is, the susceptibility gradients are in the same direction, with many chemical and physical agents. Second, the differentials in cytolysis and in development have been found very generally to agree closely with differentials in respiration, where data are available, and with differentials shown by oxidation-reduction indicators. Third, KCN, which has been much used in experiments on differential susceptibility and often as a starting-point for work with other agents, is at present regarded as an inhibitor of cytochrome oxidase. Granting this, it is to be expected that susceptibility to cyanide will vary somewhat with regional differences in activity of cytochrome oxidase, regions of greater activity being in general more susceptible than those of less activity. Comparing susceptibility to cyanide with respiration in various forms, this appears to be the case. If cytochrome oxidase is as important in general metabolism of aerobic forms as seems to be generally believed, regional differences in susceptibility to cyanide may be a rough index of quantitative regional differences in oxidative metabolism, particularly in the earlier developmental stages and simpler organisms, or wherever specific or qualitative regional differences are not far advanced. Fourth, when susceptibility to other agents, both chemical and physical, including in many cases ultraviolet radiation, X-rays, radium, excess of CO_2, and lack of oxygen, shows the same differentials and these are similar to those obtained with cyanide, it is evident that, whatever the particular actions of the different agents, the differences in susceptibility to them are not specifically different but are presumably based on quantitative differentials in the protoplasmic systems concerned. Fifth, the evidence indicates that, so far as quantitative factors are concerned, a more active protoplasmic system will be more susceptible than a less active one to any external agent which makes impossible continuation of activity at the earlier level or even continued existence of the system by action on one or more of the chemical or physical factors essential

[29] Some of these data have been mentioned above. For others up to 1940 see Child, 1941*a*, chap. iv, and for more recent data Child, 1941*b*, 1942, 1943*a*, *b*, *c*, *d*, 1945*a*, *b*.

to its activity. The activity is metabolism. With ranges of concentration or dosage of the external agent which are gradually toxic or lethal, regional differences in susceptibility become evident. Sixth, with certain ranges of concentration or dosage which are slightly or very slowly toxic, evidences of differential tolerance in different regions of the individual often appear, and with long-continued exposures a differential acclimation occurs. Regions or systems, which, according to other evidence, are more active, show greater tolerance or more rapid acclimation within certain limits than less active regions or systems. Seventh, within certain limits of concentration, dosage, and exposure period, differential recovery occurs after return to natural environment, the more active region or system recovering more rapidly than the less active. These differences in susceptibility to external agents have been shown to parallel differences in respiration or in oxidation-reduction of indicators in various organisms; they show definite relations to axiate pattern, and the conclusion seems to be justified that they constitute evidence for the presence of quantitative differentials in metabolic activity as essential factors in axiate pattern. With progress of individual development and evolution, susceptibilities of regions and organs may become specific.

In so far as axial and regional differences in susceptibility are not specific for different agents, it is to be expected that within certain ranges of concentration or dosage different agents will produce similar differential effects in retarding or otherwise modifying development. It is a well-known fact that, from the earlier attempts to modify development by means of external agents up to the present, investigators have very generally found that similar modifications are produced by different agents which cannot be supposed to act in the same way on the protoplasmic systems concerned. Moreover, the modifications have usually been found to be differential, that is, graded in relation to polarity and symmetry. To mention only a single example, essentially similar mediolateral differential inhibitions of development have been produced by different investigators and with a variety of agents in planarians, sea-urchin larvae, fishes, amphibians, and birds; and the same modifications occur in man and other mammals in teratological development.

All degrees of decrease of polarity and ventrodorsality to complete obliteration of axiate pattern have been brought about in sea-urchin development by differential susceptibility to cyanide and to other agents. Ventrodorsality disappears and the larvae become completely radial while polarity is still present. The differential inhibition resulting in complete obliteration of axiate pattern is progressive from the apical and median ventral regions, with increasing toxic effect of the agents, and these regions show the highest rates of oxidation-reduction with indicators. The apolar sea-urchin larvae are completely spherical, do not develop further, even after return to sea water, but often remain alive longer than those that develop normally (Child, 1916, 1941*a*, pp. 202 and 203, Fig. 76, *C*). Decrease and obliteration of polarity in a coelenterate planula by various agents was described above (p. 98). Even LiCl, regarded by some as regionally highly specific in action, can obliterate axiate pattern in coelenterate and sea-urchin larvae. All visible evidence of dorsiventrality has been obliterated in the frog embryo by

LiCl (Bellamy, 1919; Child, 1941*a*, Fig. 105, *A*).

With differential tolerance and differential recovery after temporary exposure, and sometimes with differential acclimation after a slight differential inhibition, differential modifications of development opposite in character to differential inhibition may gradually appear. In these modifications regions most inhibited by the more extreme effects become relatively overdeveloped; that is, polarity and ventrodorsality or dorsiventrality are exaggerated.

In general, the differential developmental modifications are characterized by graded differences in relative and absolute rates and amounts of growth and degrees of differentiation. There is little or no definite evidence of specific regional effects of the agents in many forms. In eggs with highly unequal distribution of yolk, regions of high yolk content may be highly susceptible to certain fat-soluble agents. Even in so-called "mosaic eggs" of polychete annelids, slight differential modifications have been obtained (Child, 1917). This is not regarded as proving or suggesting absence of regional specific differences, even in early developmental stages of such eggs, but merely as indicating that quantitative differentials are also present and concerned in development. Further experiment along these lines with eggs and embryos of this type is desirable. At present the only justifiable conclusion on the basis of the evidence seems to be that the differential developmental modifications are expressions of quantitative differentials in metabolism. Since they involve decrease and even complete obliteration of polarity and symmetry, on the one hand, and exaggeration of axiate pattern, on the other, it appears difficult to avoid the conclusion that in the organisms and stages concerned quantitative metabolic differentials, with, of course, corresponding differentials in the protoplasmic substrate, are the fundamental characteristics of axiate pattern.

Failure to determine effects of wide ranges of concentration or dosage and of exposure periods and to distinguish between modifications resulting from direct effect of an agent and those resulting from the secondary effects, differential tolerance, acclimation, and recovery has led some investigators to regard certain modifications as direct specific effects of particular agents. For example, the most characteristic effects of echinoderm exogastrulation do not appear during exposure to LiCl but during differential recovery from these effects, and similar modifications can be produced by other agents (Child, 1941*a*, chap. vi). The "animalizing" effect on development of exposure of unfertilized eggs to sodium thiocyanate does not occur during the exposure but only after fertilization and recovery. Exposure to thiocyanate after fertilization results in differential inhibition, often with exogastrulation; but here, as with LiCl, the extreme forms of exogastrulation appear only after return to sea water.

Differential susceptibility in relation to differential cytolysis, differential modification of development, and other lines of evidence bearing on axiate pattern has been fully discussed elsewhere (Child, 1941*a*, chaps. iv–vii). Needham (1942, p. 605) maintains that no evidence has been presented to justify "belief in existence of 'respiratory' or 'metabolic' gradients in embryos and little evidence as regards adults." He refers to the case of the amphibian "primary organizer" as showing the same oxygen uptake as ventral ectoderm and as a test case for

the respiratory gradient. The data on respiratory determinations summarized in this paper are, at present, far from agreement with Needham. The determinations of oxygen uptake by Boell, Needham, *et al.* are without definite significance, as Barth pointed out, because they take no account of differences in yolk content in the regions used for determinations. The directly visible reduction differentials in intact amphibian embryos constitute direct evidence of existence of gradients and are in essential agreement with Brachet's data on sulphydryl proteins and with a considerable part of the data on oxygen uptake. They also agree very closely with Bellamy's data on differential susceptibility in the frog embryo. If the amphibian embryo were to be regarded as a test case for the respiratory gradient, it would scarcely support Needham's conclusions, as the evidence stands at present. As already noted, some of the determinations of oxygen uptake are without definite significance; and, at best, the various determinations do not agree. Actually, however, the amphibian embryo is no more a test case for presence or absence of metabolic gradients than any other organism. It has never been maintained that axiate pattern consists only of quantitative metabolic gradients in all organisms at all stages of development. It has merely been held, on the basis of a considerable body of evidence, that axiate pattern in its simplest terms consists of such gradients; that experimental evidence demonstrates or indicates the presence of such gradients in the earlier stages of development of many organisms and persistence of quantitative differentials, even after considerable regional differentiation, in adult stages of some of the less highly differentiated organisms; that polarity and symmetry can be obliterated in some forms by inhibitors of metabolism; and that polarity and symmetry can be determined in various organisms by purely quantitative external differentials. Needham has not advanced any evidence controverting these conclusions. He dismisses differential susceptibility in a footnote (1942, p. 605) with mention of "the time taken for a piece of worm to dissolve in KCN," and the only data on respiration mentioned by him are determinations of oxygen uptake on an earthworm by Malœuf (1936). It is perhaps worth noting here that these determinations were made on extremely short pieces of body wall and that it was called to Malœuf's attention before publication that the data were without definite significance because no attempt had been made to determine the effects of isolation and preparation of the pieces, involving removal of viscera and, of course, stoppage of circulation and loss of blood. However, he preferred to publish without any attempt at control. The assumption that oxygen uptake remains unchanged in such pieces is unjustified. If a gradient pattern was originally present, it may have been completely obliterated or even reversed by differential susceptibility to the treatment in preparation. A very definite gradient pattern of oxygen uptake, decreasing from the head and increasing again posteriorly from older to younger segments, was found in *Lumbriculus* (Hyman and Galigher, 1921) and in *Nereis* (Hyman, 1932). Parker's CO_2 determinations on a planarian and *Nereis* and Shearer's data on oxygen uptake of planarians have been discussed elsewhere (Child, 1941*a*, pp. 110, 122, 730). Reduction gradient patterns similar to the respiratory gradients in *Lumbriculus* have been demonstrated in a number of

microdrilous oligochetes and several species of earthworms (Child and Rulon, 1936); and, more recently, oxidation-reduction indicator gradients have been shown to be present in the primitive oligochete *Aeolosoma* in definite relation to the appearance of new zooids in fission, and an almost identical pattern in relation to fission in two species of the rhabdocoel *Stenostomum* (Child, unpublished). Respiratory and oxidation-reduction indicator gradients in planarians and coelenterates have been referred to above. It may be noted that the indicator gradients in coelenterates concern primarily the ectoderm and in turbellarians and annelids the body wall. Gradients in the digestive tracts are apparently slight.

In the same footnote (1942, p. 605) Needham defines metabolism as "the sum total of all the chemical changes going on in the body" and holds that "the school of Child cannot but plead guilty to the charge of having a concept of metabolism too wide and vague." The school of Child, if it exists (Child does not approve of schools in this sense), is entirely innocent of any such crime. In the work on differential susceptibility, metabolism has been conceived in exactly the terms stated by Needham, and oxidation and reduction have been regarded as important components of it. On the basis of Needham's and our definition of metabolism, it appears that the oxidation-reduction gradients of developmental stages, the gradients of oxygen uptake and CO_2 production in various animals, the pigment gradient and the yolk gradient of the amphibian egg, and the yolk gradients of many other eggs constitute direct or indicative evidence of the presence of metabolic gradients. Moreover, the data on differential susceptibility, including the differential modifications of development with differential decrease and obliteration of axiate pattern, on the one hand, and exaggeration of pattern, on the other, as well as the time taken "for a piece of worm to dissolve" constitute a highly consistent body of evidence which presents considerable difficulties to interpretation in any other than metabolic terms. This evidence Needham has completely ignored.

To quote again from Needham: "Although the regularities that are established at the level of experimental morphology are irrefragible they will, in the absence of biochemical experimentation, remain forever meaningless" (1942, p. xv). Aside from the question whether anything "completely irrefragible" can remain forever meaningless, it may be noted that this somewhat oracular pronouncement is a personal opinion, not a reasoned scientific conclusion. There are meanings and meanings, and not all of them are biochemical; and even when they are biochemical, they do not always lead to hypotheses concerning specific substances. It is interesting, although it seems somewhat lacking in consistency, that the author of this statement should be so completely antagonistic to the conclusion based on various lines of direct and indirect biochemical evidence that quantitative metabolic differentials are essential factors of axiate pattern, at least in its earlier stages, and should himself postulate, without any positive evidence, a nonchemical basis for such pattern, the paracrystalline state. This basis seems at present scarcely open to biochemical experiment.

The experimental data bearing on the problem of axiate pattern constitute a body of evidence based on different lines of investigation, some of which are indicative rather than demonstrative.

However, the evidence shows a rather remarkable degree of agreement and consistency. The objection to the concept of a quantitative gradient as the initiating factor in axiate pattern, or the simplest form of such pattern, on the ground that such a gradient pattern cannot account for the origin of specific or qualitative differences appears to be unjustified. Oxygen gradients and other purely quantitative differentials have been shown to be organizers or reorganizers, and polarity and symmetry can be completely obliterated by differential susceptibility to a single agent—e.g., KCN, alcohol, ether, etc. There seem to be no theoretical difficulties as regards origin of qualitative differences at different levels of a primarily quantitative gradient. In fact, it appears doubtful that a gradient can remain completely quantitative for any considerable period in systems so complex as living protoplasms. Differences in concentration of metabolites, electrolytes, nutritive substances, and their products, in pH, in electric potential, in the colloid substrate, and conditions determining activation of different enzymes, and doubtless other differences, must arise almost immediately in a metabolic gradient. These differences, or some of them, afford conditions for activation of different genetic potentialities at different gradient-levels. The dominance of the high region of such gradients, whether it results from electrical or other dynamic factors, or from production of a substance or substances in larger amounts than in other regions, constitutes another factor, which finds expression in various organizers and reorganizers.

Although Needham rejects the view that graded differences in metabolism are essential factors in axiate pattern with little attention to the evidence, the experimental data may be permitted to speak for themselves. Also, it is of interest to note that the body of highly consistent evidence on which the concept of metabolic gradients is based stands in the sharpest contrast to the complete lack of any positive or real evidence in support of Needham's opinion that the paracrystalline state or some other molecular structure is the basis of polarity (Needham, 1942, pp. 656–77). He mentions various lines of evidence indicating or demonstrating definite molecular orientations or paracrystalline structures; but most of them concern highly differentiated protoplasms, membranes, fibers, etc. He advances certain hypothetical interpretations and suggestions and on the basis of these apparently takes it for granted that a paracrystalline or other molecular structure constitutes the primary axiate pattern. However, his discussion of polarity amounts only to the belief or faith that someday the essential structure will be discovered.

Needham says, with reference to the amphibian egg: "There is no reason for denying the maternal organism the power to construct a paracrystalline aggregate with quite complex symmetry properties." It seems only reasonable to point out that, at present, there is absolutely no reason for assuming any such power. In a footnote to this statement he says further: "In one group of animals at any rate, the insects, we have known since the work of Hallez that the egg's axial orientation is exactly the same as that of the maternal organism in the ovaries of which it was formed" (Needham, 1942, p. 218). Hallez (1886) reported this relation for certain insects; but, without questioning his observations, the fact remains that it is by no

means generally true in insects or in any other animal group. A moment's examination of the ovaries of *Drosophila*, of the housefly, or of many other insects would have shown Needham that not even the axis of the ovaries is in the same direction as the longitudinal axis of the maternal insect and that no two eggs in an ovary are in the same relation to the parental axiate pattern (Child, 1942, 1943*b*). In animal ovaries generally, the axes of ovarian oöcytes are in all possible positions with relation to the maternal axiate pattern. In the sea urchin, the starfish, and some other animals the axiate pattern of the adult is widely different from that of the embryo and larva.

As already noted, the problem of the origin and nature of polarity and of axiate pattern in general concerns not merely single cells but multicellular systems, such as the *Tubularia* and *Corymorpha* pieces and cell aggregates, planarian and annelid pieces, appendages of various animals, including amphibians, and in embryonic development, blastulae and planulae of coelenterates, sea-urchin blastulae and gastrulae, and, so far as axiate organ systems are concerned, many other developmental stages. With the great number of membranes in these systems in all possible spatial relations and the probable orientation of molecules in relation to them and in fibrillar and other intracellular structures and the cell movements during development, it is somewhat difficult to conceive a definite, static molecular structure extending through the whole system and persisting as an axiate pattern. Moreover, recent investigations indicate that protoplasmic components very generally are undergoing continuous chemical change. If true, this does not seem to favor the molecular hypothesis of axiate pattern. Can a low concentration of cyanide, alcohol, ether, etc., destroy such a molecular pattern; and can an external oxygen gradient and various other external differentials determine a new one? Would not obliteration of ventrodorsality, with persistence of polarity in the sea-urchin blastula or gastrula, or determination of a dorsiventral pattern in protoplasms that are normally radial present difficulties of interpretation in terms of a paracrystalline or other molecular axiate pattern?

In the course of his discussion of polarity Needham calls attention to the "rigidity" of polarity in animal eggs. There is, perhaps, a simple reason for such rigidity as exists. Most experimental procedures do not greatly disturb the egg cortex or the colloid substrate; and the gradient pattern may continue unchanged, even though solid inclusions are displaced, as in centrifuging. Polarity is not rigid in inverted amphibian eggs in which both material and metabolic pattern are evidently disturbed and altered. There is perhaps less rigidity in multicellular systems because a metabolic differential may extend from cell to cell. On the basis of the molecular hypothesis, it is not evident why the rigidity should be less in a multicellular than in a unicellular system.

The hypothesis or belief that axiate pattern of organisms is based on a preformed molecular orientation or structure of some sort and that metabolism is of secondary importance is an interesting feature of present-day biology. Paracrystalline states and other molecular orientations doubtless occur widely in living organisms, but it has not yet been shown that they precede and determine metabolic differentials. The evidence at present available seems to in-

dicate that they become increasingly evident and probably increasingly important with progress of development, structurization, and differentiation. If they are concerned in such features of development as asymmetry, which is usually not evident at the beginning of development, it seems probable that they are consequences of the characteristics of metabolism in synthesis of protein and perhaps of other molecules. Metabolism, of course, involves a protoplasmic substrate in which enzymes are essential features. The evidence at hand, as opposed to personal opinion, indicates gradients of enzyme activity, associated with various other molar or regional, rather than molecular, differentials, as a more probable and directly effective basis for spatial developmental pattern than any sort of molecular structure. Axiate pattern decreases and disappears with decreasing size of isolated pieces of various animals. It has not yet been made clear how a static molecular structure can account for the following characteristics of development: first, the regional features and order of axiate pattern and morphogenesis in multicellular systems as well as in single cells; second, the differential modifications of development resulting from general exposure to external chemical and physical agents, involving the progressive differential decrease and the obliteration of axiate pattern by agents inhibiting metabolism directly or indirectly and exaggerations of pattern in differential recovery and, to some extent, in differential tolerance and acclimation or conditioning; third, determination of axiate patterns, often very different from the original, by purely quantitative external differentials which influence metabolism; and, fourth, alteration of localization of parts, that is, increase or decrease of scale of organization, by general exposure to external agents.

Criticisms of the gradient concept by Brachet (1945) are particularly concerned with proving that the gradients, if present at all, are not respiratory gradients. That these criticisms are misdirected should be evident from an earlier consideration of methods of physiological analysis (Child, 1941*a*, chap. iii). On page 76 of this chapter appears the following: "It is not necessary to assume that respiration or oxidation is always the primary factor in the various expressions of differential susceptibility." Since the gradients indicated by differential susceptibility are, in the great majority of cases, the same as those indicated by oxidation-reduction indicators and in developmental stages by differentials in development and growth, the foregoing statement holds for these differentials also. However, in some cases respiratory gradients have been found to coincide with gradients of differential susceptibility and those shown by oxidation-reduction indicators.

It must also be noted that, whether the oxidation-reduction gradients do or do not give evidence of gradients in rate of oxidation or reduction, the indicator methods are far more delicate as means of bringing to light axial regional differentials than any methods at present available for determining oxygen uptake. Taking, for example, the sea-urchin embryo, Brachet concludes that there is no respiratory gradient in this embryo because Lindahl and Holter (1940) found no difference in oxygen uptake in apical and basal (animal and vegetal) halves of early developmental stages. These data may be entirely correct, but the determinations require a considerable number of embryos or halves and a considerable time, and the authors ad-

mit a 10 per cent experimental error. Also, it is not known whether changes in oxygen uptake occur after isolation of the halves. The indicator gradients appear in each intact embryo in a few minutes. In early developmental stages they are barely distinguishable, but they become more distinct as development progresses. The micro-method used by Lindahl and Holter cannot be expected to bring consistently to light differences so slight as those shown by the indicators, even if we assume that a respiratory gradient parallels the indicator gradient—an assumption that may or may not be correct. In short, negative evidence from determinations of oxygen uptake is not adequate as evidence against indicator data. The limitations of the indicator method appear to be those imposed by the ability of the human eye to distinguish differences in color. The indicator gradients certainly give evidence of differentials of some sort. The very close association of these gradients with differentials in growth and developmental activity and often in functional activity in fully developed individuals makes it highly improbable that they are entirely independent of metabolic differentials.

The differential modifications of embryonic and reconstitutional development, which are completely ignored by both Needham and Brachet, are, to a high degree, nonspecific in character with many different agents which cannot be assumed to act on living protoplasms in exactly the same ways. They also show similar axial relations in many respects in coelenterates, planarians, echinoderms, and vertebrates. With increasing effect of agents inhibiting development—for example, KCN, LiCl—ventrodorsality and polarity, as indicated by axiate development, decrease progressively to complete obliteration of axiate pattern in sea-urchin development, even after gastrulation. Progressive obliteration of axiate pattern in development of a coelenterate was shown above in Figure 7. The regions most inhibited in these cases correspond very closely to regions of most rapid oxidation and reduction of indicators. Unless we assume that development takes place without metabolism, it is difficult to account for these developmental modifications except in relation to essentially quantitative metabolic differentials of some sort.

On the other hand, it was pointed out above that polarity and symmetry can be determined in various organisms by purely quantitative external differentials. It has been found that indicator gradients and susceptibility gradients result from action of these differentials. Since Needham and Brachet, in their criticisms of the gradient concept, have completely ignored a large body of data which in certain respects are highly significant for the gradient problem, it has seemed necessary to point out once more the bearing of these lines of experiment on the problem.

Brachet (1945, e.g., p. 396) is completely in error in stating that Child regards the gradients as exclusively quantitative. It has been repeatedly stated that they are regarded as quantitative only in origin and in their simplest terms and that they probably do not remain entirely quantitative for any considerable time, although quantitative factors may persist and play important parts, even throughout life in some organisms—more particularly in those which are not highly differentiated.

Neither Needham nor Brachet has presented positive evidence for any other concept of axiate pattern. As pointed out

above, Needham apparently assumes that the paracrystalline state is the basis of polarity and symmetry and that this state is determined by the parent organism, but he presents no positive evidence for this opinion. Brachet's criticisms are essentially negative: he offers nothing in place of the gradient concept of axiate pattern.

CONCLUSION AND SUMMARY

If the organizer concept is to be of any real value in analysis of developmental phenomena, it must not be based on amphibian development alone but must be broad enough to include all kinds of development. Interesting and important as the experimental data concerning the amphibian "primary organizer" are, they do not provide a basis for analysis of development in general; nor do they, as they stand at present, throw any appreciable light on the general problem of organization. The so-called "primary organizer" is not primary, it does not determine ectodermal or other axiate pattern in natural development; in at least some amphibians it is not absolutely necessary for neural differentiation; and it has not yet been proved to be specific in action or to differ specifically at different levels. At best it represents only a step or steps in the progressive organization and development of the amphibian embryo. It is a differentiator, rather than a primary organizer, in natural development. During recent years amphibian development and particularly amphibian organizer data and problems have monopolized attention to such a degree that various other aspects and problems of developmental physiology have remained more or less in the background, even in some textbooks; and theories of development and of organizers of limited scope and value have resulted. Amphibian development is by no means a primitive type of development, nor does it begin with gastrulation. Even the unfertilized egg is organized, and the primary organizer is undoubtedly external to the oöcyte but is not the axiate pattern of the parent.

A cell or cell mass cannot exist without at least a surface-interior organization, resulting from the presence about it of an external medium which determines an ectoplasm, a plasma membrane, or some other difference between surface and interior, and to that extent is an organizer. But something more is necessary for development of the cell into an axiate organism. Viewed in the light of development in general, reconstitutional, as well as embryonic, development, an organizer is a determiner or inductor of spatial developmental pattern which becomes evident physiologically and in the regional pattern of morphogenesis and differentiation. This pattern may be induced in a single cell, e.g., the egg, or in a multicellular system, such as a cell aggregate of *Corymorpha* and other hydroids, a blastula, a gastrula, or isolated pieces of organisms.

An organizer may determine axiate pattern in a cell or cell aggregate which previously had nothing but a surface-interior organization, or it may be a reorganizer and alter a pre-existing axiate pattern in embryonic or adult reconstitution. Organizers are, in general, external to the individual or part organized or reorganized and may be completely extraorganismic. Experimental evidence indicates that organizers concerned with the beginnings and earlier stages of axiate development are primarily determiners or inductors of gradient patterns involving metabolic differentials. Purely quantitative extraorganismic differentials which influence

metabolic rate are often organizers or reorganizers. Whether, or to what extent, intraorganismic organizers do more than determine gradient patterns, particularly in later developmental stages, remains to be determined. The inductors of particular organ systems and organs, for example, the amphibian lens, balancer, otic vesicle, and even the leg, are apparently not actually organizers at all but merely inductors. It seems to be generally conceded that the specificity of effect, that is, the organ system or organ which develops, in these inductions, depends on the reacting system. It is at least open to question whether these inductors are primarily anything more than activators.

The experimental evidence at present available, as opposed to opinion, points to the conclusion that axiate pattern in its beginnings and simplest forms consists in graded quantitative differentials in general metabolism, associated with graded enzyme and other differentials in the protoplasm. In general, intraorganismic reorganizers in reconstitutional development, such, for example, as the developing hydranth of *Corymorpha* or the developing planarian head, are regions of relatively high metabolic activity and exercise their reorganizing activity on lower gradient-levels or on regions without definite axiate pattern. Since the differential modifications of embryonic and reconstitutional development resulting from general exposure to external chemical and physical agents show little or no evidence of specific difference in character with different agents, they indicate that the factors concerned are primarily quantitative differentials of some sort; and since they involve alteration of differentials in rate of development, growth, differentiation, and absolute and relative sizes of parts, it appears beyond question that their essential characteristics are determined directly or indirectly by quantitative differentials in metabolism. Moreover, since all evidence of polarity and symmetry can be obliterated in these differential modifications, and new polarities and symmetries can be determined by external differentials which influence rate of metabolism, the only logical conclusion from the data seems to be that quantitative metabolic differentials or gradients are fundamental factors of polarity and symmetry in their beginnings and their simplest forms.

This paper is to such an extent a summary of a great volume of investigation that any summary other than these general conclusions seems unnecessary and merely repetitious.

LITERATURE CITED

BARTH, L. G. 1934. The effect of constant electric current on the regeneration of certain hydroids. Physiol. Zoöl., 7:340.

———. 1937. Chemical stimulation of the amphibian ectoderm. Biol. Bull., 73:346.

———. 1938. Oxygen as a controlling factor in the regeneration of *Tubularia*. Physiol. Zoöl., 11:179.

———. 1939*a*. The chemical nature of the amphibian organizer. III. *Ibid.*, 12:22.

———. 1939*b*. Neural differentiation without organizer. Biol. Bull., 77:299 (abstract).

———. 1939*c*. Neural differentiation without organizer. Anat. Rec., Suppl., 75, No. 4, 116 (abstract).

———. 1940*a*. The relation between oxygen consumption and regeneration. Biol. Bull., 78:366.

———. 1940*b*. The process of regeneration in hydroids. Biol. Rev., 15:405.

———. 1941. Neural differentiation without organizer. Jour. Exper. Zoöl., 87:371.

———. 1942. Regional differences in oxygen consumption of the amphibian gastrula. Physiol. Zöol., 15:30.

———. 1944. The determination of the regenerating hydranth in *Tubularia*. *Ibid.*, 17:355.

BARTH, L. G., and GRAFF, S. 1938. The chemical nature of the amphibian organizer. Cold Spring Harbor Symp. on Quant. Biol., 6:385.

BEATTY, R. A.; DE JONG, S.; and ZIELINSKI, M. A. 1939. Experiments on the effect of dyes in induction and respiration in the amphibian gastrula. Jour. Exper. Biol., 16:150.

BELLAMY, A. W. 1919. Differential susceptibility as a basis for control and modification of early development in the frog. Biol. Bull., 37:312.

———. 1921. Note concerning the origin of polarity in the frog's egg: a correction. *Ibid.*, 41:351.

BERRILL, N. J. 1931. Regeneration in *Sabella pavonina* (Sav.) and other sabellid worms. Jour. Exper. Zoöl., 58:495.

BERRILL, N. J., and MEES, DORIS. 1936*a*. Reorganization and regeneration in *Sabella*. I. Jour. Exper. Zoöl., 73:67.

———. 1936*b*. Reorganization, etc. II. *Ibid.*, 74:61.

BOELL, E. J. 1938. Metabolic properties of the regions of the amphibian gastrula. Anat. Rec., Suppl., 72, No. 4, 34 (abstract).

———. 1942. Biochemical and physiological analysis of organizer action. Fourth growth symposium. Growth, Suppl., 6:37.

BOELL, E. J.; KOCH, H.; and NEEDHAM, J. 1939. Morphogenesis and metabolism. IV. Proc. Roy. Soc., London, B, 127:374.

BOELL, E. J., and NEEDHAM, J. 1939. Morphogenesis and metabolism. II, III. Proc. Roy. Soc., London, B, 127:356, 363.

BOELL, E. J.; NEEDHAM, J.; and ROGERS, V. 1939. Morphogenesis and metabolism. I. Proc. Roy. Soc., London, B, 127:322.

BOELL, E. J., and NICHOLAS, J. S. 1940. Respiratory rate and yolk content of the amphibian gastrula. Anat. Rec., Suppl., 78, No. 4, 76 (abstract).

BRACHET, J. 1934*a*. Métabolisme respiratoire du centre organisateur de l'œuf de grenouille. Compt. rend. Soc. biol., 116:58.

———. 1934*b*. Etude du métabolisme de l'œuf de grenouille au cours du développement. I. Arch. de biol., 45:611.

———. 1934*c*. Etude, etc. III. *Ibid.*, 46:25.

———. 1936. Le Métabolisme respiratoire du centre organisateur de l'œuf de grenouille (*Rana fusca*). Compt. rend. Soc. biol., 122:108.

———. 1938. La Localisation des protéines sulfhydrilées pendant le développement des Amphibiens. Bull. Acad. roy. de Belgique, Cl. des sci., 24:499.

———. 1939. Etude du métabolisme, etc. V. Arch. de biol., 50:233.

———. 1945. Embryologie chimique. Paris: Masson et Cie. Pp. 1–535.

BRACHET, J., and RAPKINE, L. 1939. Oxydation et reduction d'explantats dorsaux et ventraux de gastrulas (Amphibiens). Compt. rend. Soc. biol., 131:789.

BRACHET, J. and SHAPIRO, H. 1937. The relative oxygen consumption of dorsal and ventral regions of intact amphibian gastrulae, including observations on unfertilized eggs. Jour. Cell. and Comp. Physiol., 10:133.

BRAGG, A. N. 1939. Observations upon amphibian deutoplasm and its relation to embryonic and early larval development. Biol. Bull., 77:268.

BROWNE, ETHEL. 1909. The production of new hydranths in *Hydra* by the insertion of small grafts. Jour. Exper. Zoöl., 7:1.

CHILD, C. M. 1903. Studies on regulation. III. Arch. f. Entw'mech., 17:1.

———. 1907. Analysis of form regulation in *Tubularia*. V. *Ibid.*, 24:285.

———. 1909. Factors of form regulation in *Harenactis attenuata*. III. Jour. Exper. Zoöl., 6:353.

———. 1910. Further experiments on adventitious reproduction and polarity in *Harenactis*. Biol. Bull., 19:1.

———. 1916. Experimental control and modification of larval development in the sea urchin in relation to the axial gradient. Jour. Morph., 28:65.

———. 1917. Differential susceptibility and differential inhibition in the development of polychete annelids. *Ibid.*, 30:1.

———. 1923. The axial gradients in Hydrozoa. V. Biol. Bull., 45:181.

———. 1924. Physiological foundations of behavior. New York: Henry Holt & Co. Pp. i–ix+1–330.

———. 1925*a*. The axial gradients in Hydrozoa. VI. Biol. Bull., 48:19.

———. 1925*b*. The axial gradients, etc. VII. *Ibid.*, p. 176.

———. 1926*a*. Studies on the axial gradients in *Corymorpha palma*. II. Biol. generalis, 2:609.

———. 1926*b*. Studies, etc., III. *Ibid.*, p. 771.

———. 1927*a*. Modifications of polarity and symmetry in *Corymorpha palma* by means of inhibiting conditions and differential exposure. II. Jour. Exper. Zoöl., 47:343.

———. 1927*b*. Experimental localization of new axes in *Corymorpha* without obliteration of the original polarity. Biol. Bull., 53:469.

———. 1928. Axial development of aggregates of dissociated cells from *Corymorpha palma*. Physiol. Zoöl., 1:419.

———. 1929*a*. Lateral grafts and incisions as organizers in the hydroid, *Corymorpha*. *Ibid.*, 2:342.

———. 1929*b*. Physiological dominance and physiological isolation in development and reconstitution. Arch. f. Entw'mech., 117:21.

———. 1932. Determination of new axes by lateral grafts and incisions in *Corymorpha* in relation to the gradient and dominance. Physiol. Zoöl., 5:593.

———. 1935. Dominance of hydranths induced by grafts in *Corymorpha*. Jour. Exper. Zoöl., 71:375.

———. 1941*a*. Patterns and problems of development. Chicago: University of Chicago Press. Pp. i–ix+1–811.

———. 1941*b*. Formation and reduction of indo-

phenol blue in development of an echinoderm. Proc. Natl. Acad. Sci., 27:523.

———. 1942. Differential dye reduction in ovaries of *Drosophila hydei*. Physiol. Zoöl., 15:13.

———. 1943*a*. Differential dye reduction and re-oxidation in *Triturus* development. *Ibid.*, 16:81.

———. 1943*b*. Indophenol reaction and reduction in living ovaries of *Drosophila hydei*. *Ibid.*, p. 141.

———. 1943*c*. Gradient pattern in developing gill, leg, and balancer of *Triturus*. *Ibid.*, p. 287.

———. 1943*d*. Oxidation-reduction pattern in development of a teleost. *Ibid.*, p. 297.

———. 1944. Developmental pattern in the starfish, *Patiria miniata*, as indicated by indophenol. *Ibid.*, 17:129.

———. 1945*a*. Oxidation-reduction patterns in ciliate Protozoa. *Ibid.*, 18:250.

———. 1945*b*. A further study of oxidation-reduction pattern in teleost development. Jour. Exper. Zoöl., 100:577.

CHILD, C. M., and HYMAN, L. H. 1926. Studies on the axial gradients in *Corymorpha palma*. I. Biol. generalis, 2:355.

CHILD, C. M., and RULON, O. 1936. Differential reduction of vital dyes in certain oligochetes. Jour. Exper. Zoöl., 74:427.

CHILD, C. M., and WATANABE, Y. 1935. Differential reduction of methylene blue by *Corymorpha palma*. Physiol. Zoöl., 8:395.

CHUANG, H. H. 1938. Spezifische Induktionsleistungen von Leber und Niere. Biol. Zentralbl., 58:472.

———. 1940*a*. Induktionsleistungen von frischen und gekochten Organteilen (Niere, Leber) nach ihren Verpflanzungen in Explantaten und verschiedenen Wirtsregionen von Tritonkeimen. Arch. f. Entw'mech., 139:556.

———. 1940*b*. Weitere Versuche über die Veränderung der Induktionsleistung von gekochten Organteilen. *Ibid.*, 140:25.

COE, W. R. 1932. Regeneration in nemerteans. III. Jour. Exper. Zoöl., 61:29.

———. 1934*a*. Regeneration in nemerteans. IV. *Ibid.*, 67:283.

———. 1934*b*. Analysis of the regenerative process in nemerteans. Biol. Bull., 66:304.

COHEN, A. 1938. Induction by cauterization in the amphibian egg. Collecting Net, 13:87.

DALCQ, A. 1938. Form and causality in early development. Cambridge University Press.

———. 1940. Contribution à l'étude du potentiel morphogénétique chez les Anoures. I. Arch. de biol., 51:387.

DALCQ, A., and PASTEELS, J. 1938. Potentiel morphogénétique, régulation et "axial gradients" de Child. Bull. Acad. roy. med. de Belgique, sér. 6, 3:261.

FISCHER, F. G., and HARTWIG, H. 1936. Die Vitalfärbung von Amphibienkeimen zur Untersuchung ihrer Oxydations-Reduktions-Vorgänge. Zeitschr. f. vergl. Physiol., 24:1.

———. 1938. Vergleichende Messungen der Atmung des Amphibienkeimes und seiner Teile während der Entwicklung. Biol. Zentralbl., 58:568.

GROSS, F., and HUXLEY, J. S. 1935. Regeneration and reorganization in *Sabella*. Arch. f. Entw'mech., 133:582.

HALLEZ, P. 1886. Loi de l'orientation de l'embryon chez les Insectes. Compt. rend. Acad. sci., 103:606.

HEATLEY, N. G.; WADDINGTON, C. H.; and NEEDHAM, J. 1937. Studies on the nature of the amphibian organization centre. VI. Proc. Roy. Soc., London, B, 122:403.

HÖRSTADIUS, S. 1935. Über die Determination im Verlaufe der Eiachse bei Seeigeln. Pub. Staz. zool. Napoli, 14:251.

———. 1936*a*. Über die zeitliche Determination im Keim von *Paracentrotus lividus* Lk. Arch. f. Entw'mech., 135:1.

———. 1936*b*. Weitere Studien über die Determination im Verlaufe der Eiachse bei Seeigeln. *Ibid.*, p. 40.

HOLMES, S. J. 1944. The problem of organic form. I–IV. Sci. Monthly, 59:226, 255, 379.

HOLTFRETER, J. 1933*a*. Der Einfluss von Wirtsalter und verschiedenen Organbezirken auf die Differenzierung von angelagertem Gastrulaektoderm. Arch. f. Entw'mech., 127:619.

———. 1933*b*. Organisierungsstufen nach regionaler Kombination von Entomesoderm mit Ektoderm. Biol. Zentralbl., 53:403.

———. 1934*a*. Der Einfluss thermischer, mechanischer und chemischer Eingriffe auf die Induzierbarkeit von *Triton*-Keimteilen. Arch. f. Entw'mech., 132:225.

———. 1934*b*. Über die Verbreitung induzierender Substanzen und ihre Leistungen im Tritonkeimen. *Ibid.*, p. 307.

———. 1944. Neural induction of ectoderm through exposure to salt solution. Jour. Exper. Zoöl., 95:307.

———. 1945. Neuralization and epidermization of gastrula ectoderm. *Ibid.*, 98:161.

HUXLEY, J., and DE BEER, G. R. 1934. Elements of experimental embryology. Cambridge University Press. Pp. i–xii+1–514.

HYMAN, L. H. 1916. An analysis of the process of regeneration in certain microdrilous oligochetes. Jour. Exper. Zoöl., 20:99.

———. 1920. The axial gradients in Hydrozoa. III. Biol. Bull., 38:353.

———. 1921. The metabolic gradients of vertebrate embryos. I. *Ibid.*, 40:32.

———. 1923. Physiological studies on *Planaria*. V. Jour. Exper. Zoöl., 37:47.

———. 1926. The axial gradients in Hydrozoa. VIII. Biol. Bull., 50:406.

———. 1927. The metabolic gradients of vertebrate embryos. III. *Ibid.*, 52:1.

———. 1932. The axial respiratory gradient: experimental and critical. Physiol. Zoöl., 4:566.

HYMAN, L. H., and GALIGHER, A. E. 1921. Direct demonstration of the existence of a metabolic gradient in annelids. Jour. Exper. Zoöl., 34:1.

JAEGER, LUCENA. 1945. Glycogen utilization by the amphibian gastrula in relation to invagination. Jour. Cell. and Comp. Physiol., 25:97.

LEHMANN, F. E. 1938. Regionale Verschiedenheiten des Organisators von *Triton*, etc. Arch. f. Entw'mech., 138:106.

LINDAHL, P. E., and HOLTER, H. 1940. Die atmung animaler und vegetativer Keimhälften von *Paracentrotus lividus*. Compt. rend. trav. lab. Carlsberg, sér. chim., 23:257.

LUND, E. J. 1921. Experimental control of organic polarity by the electric current. I. Jour. Exper. Zoöl., 34:471.

LUTHER, W. 1935. Entwicklungsphysiologische Untersuchungen am Forellenkeim. Biol. Zentralbl., 55:114.

———. 1936*a*. Potenzprüfung an isolierten Teilstücken der Forellenkeimscheibe. Arch. f. Entw'mech., 135:359.

———. 1936*b*. Austausch von präsumptiver Epidermis und Medullarplatte bei Forellenkeim. *Ibid.*, p. 384.

———. 1937. Transplantations- und Defektversuche am Organisationszentrum der Forellenkeimscheibe. *Ibid.*, 137:435.

MALŒUF, N. S. R. 1936. The "metabolic" gradient in the adult earthworm. Biol. Zentralbl., 56:429.

MILLER, J. A. 1938. Studies on heteroplastic transplantation in triclads. Physiol. Zoöl., 11:214.

———. 1939. Experiments on polarity determination in *Tubularia* regeneration. Anat. Rec., Suppl., 75, No. 4, 38 (abstract).

———. 1942. Some effects of covering the perisarc upon tubularian regeneration. Biol. Bull., 83:416.

MUTZ, ELFRIEDA. 1930. Transplantationsversuche an *Hydra* mit besonderer Berücksichtigung der Induktion Regionalität und Polarität. Arch. f. Entw'mech., 121:210.

NEEDHAM, J. 1938. Morphogénèse et métabolisme des hydrates de carbone. Trav. du Congrès du Palais de la découverté, Paris, 1938.

———. 1939. Biochemical aspects of organizer phenomena. First symposium on development and growth. Growth, Suppl., 1939, p. 45.

———. 1942. Biochemistry and morphogenesis. Cambridge University Press. Pp. i–xvi+1–785.

NEEDHAM, J., and BOELL, E. J. 1938. Metabolic properties of the regions of the amphibian gastrula. Proc. Soc. Exper. Biol. and Med., 39:287.

NEEDHAM, J.; ROGERS, V.; and SHEN, S. C. 1939. Morphogenesis and metabolism. V. Proc. Roy. Soc., London, B, 127:576.

NEEDHAM, J.; WADDINGTON, C. H.; and NEEDHAM, D. M. 1934. Physico-chemical experiments on the amphibian organizer. Proc. Roy. Soc., London, B, 114:393.

OKADA, Y. K. 1938. Neural induction by inorganic matters. Mem. College of Sci., Kyoto Imp. Univ., B, 14:301.

OKADA, Y. K., and SUGINO, H. 1937. Transplantation experiments in *Planaria gonocephala* Duges. Japanese Jour. Zoöl., 7:373.

OPPENHEIMER, JANE M. 1936*a*. The development of isolated blastomeres of *Fundulus heteroclitus*. Jour. Exper. Zoöl., 72:247.

———. 1936*b*. Transplantation experiments on developing teleosts (*Fundulus* and *Perca*). *Ibid.*, p. 409.

———. 1936*c*. Processes of localization in developing *Fundulus*. *Ibid.*, 73:405.

———. 1938. Potencies for differentiation in the teleostean germ ring. *Ibid.*, 79, 185.

PARKER, G. H. 1929. The metabolic gradient and its applications. Brit. Jour. Exper. Biol., 6:412.

PASTEELS, J. 1938*a*. Résultats expérimentaux concernant le déterminisme précoce chez les Amphibiens. Compt. rend. Assoc. anat., 1938, p. 1.

———. 1938*b*. Recherches sur les facteurs initiaux de la morphogénèse chez les Amphibiens. I. Arch. de biol., 49:629.

———. 1939. Recherches, etc. II. *Ibid.*, 50:291.

———. 1940. Recherches, etc. III. *Ibid.*, 51:103.

PIEPHO, H. 1938. Über Oxidations-Reduktionsvorgänge in Amphibienkeim. Biol. Zentralbl., 58:90.

ROBBINS, HARRIET, and CHILD, C. M. 1920. Carbon dioxide production in relation to regeneration in *Planaria dorotocephala*. Biol. Bull., 38:103.

RULON, O. 1936. Differential reduction of Janus green during development of the chick. Protoplasma, 24:346.

———. 1941. Modification of development in the sand dollar by NaCNS and Ca-free sea water. Physiol. Zoöl., 14:305.

———. 1944. The control of reconstitutional development in *Dugesia dorotocephala* with ethyl alcohol. *Ibid.*, 17:152.

RULON, O., and CHILD, C. M. 1937. Experiments on scale of organization in *Euplanaria dorotocephala*. *Ibid.*, 10:396.

RUSTIA, C. P. 1925. The control of biaxial development in the reconstitution of pieces of *Planaria*. Jour. Exper. Zoöl., 42:111.

SANTOS, F. 1929. Studies on transplantation in *Planaria*. Biol. Bull., 57:188.

———. 1931. Studies, etc. Physiol. Zoöl., 4:111.

SCHLEIP, W. 1929. Die Determination der Primitiventwicklung. Leipzig: Akad. Verlagsgesellsch. Pp. i–xii+1–914.

SCHULTZE, O. 1894. Die künstliche Erzeugung von Doppelbildungen bei Froschlarven mit Hilfe abnormer Gravitationswirkung. Arch. f. Entw'mech., 1:269.

SEIDEL, F. 1926. Die Determinierung der Keimanlage bei Insekten. I. Biol. Zentralbl., 46:321.

SEIDEL, F. 1928. Die Determinierung, etc. II. *Ibid.*, **48**:230.

———. 1929*a*. Die Determinierung, etc. III. *Ibid.*, **49**:547.

———. 1929*b*. Untersuchungen über die Bildungsprinzip der Keimanlage im Ei der Libelle, *Platycmenis pennipes*. I–V. Arch. f. Entw'mech., **119**:322.

———. 1935. Der Anlageplan im Libellenei. *Ibid.*, **132**:671.

SPEMANN, H. 1936. Experimentelle Beiträge zu einer Theorie der Entwicklung. Berlin: Springer. Pp. i–viii+1–296.

———. 1938. Embryonic development and induction. Yale University Press. Pp. i–xii+1–401.

SPEMANN, H., und MANGOLD, H. 1924. Über Induktion von Embryonalanlagen durch Implantation artfremde Organisatoren. Arch. f. mikr. Anat. u. Entw'mech., **100**:599.

VAN CLEAVE, C. D. 1929. An experimental study of fission and reconstitution in *Stenostomum*. Physiol. Zoöl., **2**:18.

WADDINGTON, C. H. 1934. Experiments on embryonic induction, Part II. Jour. Exper. Biol., **11**:15.

———. 1938. Morphogenetic substances in early development. Trav. du Congrès du Palais de la découverté, Paris, 1938, p. 437.

———. 1939. Genes as evocators in development. First growth symposium. Growth, Suppl., **3**:37.

WADDINGTON, C. H.; NEEDHAM, J.; and BRACHET, J. 1936. Studies on the nature of the amphibian organizer. Proc. Roy. Soc., London, B, **120**:173.

WADDINGTON, C. H.; NEEDHAM, J.; NOWINSKI, W.; LEMBERG, R.; and COHEN, A. 1936. Studies, etc. Proc. Roy. Soc., London, B, **120**:198.

WADDINGTON, C. H., and SCHMIDT, G. A. 1933. Induction by heteroplastic grafts of the primitive streak of birds. Arch. f. Entw'mech., **128**:522.

WATANABE, Y. 1935. Physiological dominance in *Corymorpha palma* in relation to reconstitution and methylene blue reduction. Physiol. Zoöl., **8**:417.

WEISS, P. 1935. The so-called organizer and the problem of organization. Physiol. Rev., **15**:639.

———. 1939. Principles of development. New York: Henry Holt & Co. Pp. i–xix+1–601.

YAO, T. 1945. Studies on the organizer problem in *Pelmatohydra oligactis*. I–III. Jour. Exper. Biol., **21**:147.

ZWILLING, E. 1939. The effect of the removal of perisarc on regeneration in *Tubularia crocea*. Biol. Bull., **76**:90.

THE THROMBOPLASTIC EFFECTS OF TISSUE EXTRACTS AND THEIR INHIBITION BY IMMUNE SERA[1]

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THE data presented in this paper resulted from preliminary experiments in which chickens were injected intravenously with intracellular stages of coccidia. When coccidia were injected, along with certain cellular elements, chickens died within 1 minute after the injection. Attempts to learn how to prevent the death of our experimental animals led to the study of tissue extracts and intravascular coagulation herewith reported.

Wooldridge (1886) described intravascular coagulation in rabbits, dogs, and cats that resulted from injections of extracts of testis and thymus secured from young calves. The injections caused almost instantaneous death, and Wooldridge identified the substance responsible as a lecithin protein compound. Lytschkowsky and Rougentzoff (1913) found that intravascular clotting and death followed injections of saline extracts of rabbit lungs into rabbits. Baker, Noran, and Larson (1945) reported similar results and also showed that homogenized beef lung was lethal to the rabbit, though the action was delayed. Again the cause of death was intravascular coagulation, and they suggested that either thrombin or prothrombin was the causative agent. Lungs of pig, sheep, guinea pig, and human, and

[1] Supported in part by a grant-in-aid from the Wisconsin Alumni Research Foundation. Published with the permission of the Wisconsin Agricultural Experiment Station.

extracts of rabbit tissue other than lung, namely, kidney, liver, spleen, brain, and nasal mucosa, were not toxic to rabbits. Mills and Matthews (1924) obtained *in vivo* coagulation and death of rabbits following an injection of freshly prepared thrombin; and Mellanby (1933) secured the same results in rabbits injected with thrombase (thrombin), prepared from oxalated ox plasma. He was unable to produce an immunity against thrombin by repeated intravenous injections of minimal doses over a period of several weeks.

Smith and Smith (1940) found that menstrual discharge contains a toxin which killed rats within 48 hours following subcutaneous injections and which was lethal to rabbits when injected in sufficiently large doses. It is also known that tissues may release substances that are toxic to laboratory animals as a result of damage or irritation. Menkin (1943) used pleural exudate from dogs treated with turpentine and found that, intravenously injected, this material caused severe toxic symptoms in dogs; when injected subcutaneously into dogs or rabbits, it produced tissue damage. He called the active principle "necrosin." Smith and Smith (1945) showed that menstrual toxin might be identical with the necrosin of pleural exudates, as determined by physical, chemical, and biological similarities as well as by cross-immunity tests. Both substances clot blood. Schloss (1920–21) reported that blood of infants suffering with a type of intestinal intoxication was toxic to guinea pigs. Boyd (1923) found that portal blood from cases of acute intestinal intoxication in children had toxic effects on guinea pigs. For additional references on tissue toxicity and toxicity of blood and sera see the extensive reviews of N. R. Smith (1919) and Mills (1921).

METHODS

The experiments to be described involve the intravenous injections of various tissue extracts. These extracts and their effects are listed in Table 1. All tissues except brain, upon extirpation from the animal's body, were washed with water or with saline solution to remove blood and extraneous materials such as those present in the lumen of the digestive tract. Brain was not so washed because it ordinarily contains very little blood and because it exhibits a marked affinity for water. The tissues, with the exception of two samples of rabbit lung, were then frozen, thawed, and homogenized. In order to facilitate the homogenizing, a volume of 1.8 per cent saline, approximately equal to the volume of the tissue, was added in each case. The material was then centrifuged, and the supernatant liquid was frozen (except the rabbit lung) and retained for

TABLE 1

EFFECTS OF TISSUE EXTRACTS ON CHICKENS AND RABBITS

Tissue Extract	No. of Extracts	Effect on—	
		Chickens	Rabbits
Chicken caeca	11	Lethal	None
Chicken lung	4	Lethal	None
Chicken brain	3	Lethal	None
Chicken testis	1	Lethal	None
Chicken digestive tract	5	Lethal	†
Chicken spleen	2	None	†
Chicken liver	2	None	†
Chicken kidney	2	None	†
Chicken pancreas	2	Sick	†
Chicken muscle	1	None	†
Rabbit lung	4	Varied*	Lethal
Rabbit caecum	1	None	†
Guinea-fowl caecum	1	Lethal	†
Pigeon lung	1	Lethal	†
Turkey caecum	2	Lethal	†
Guinea-pig digestive tract	1	None	†
Rat digestive tract	1	None	†

* See text.
† No test made.

injection. Rabbit lung was not frozen because freezing seems to cause a marked precipitation, accompanied by loss of some of the toxicity.

The rabbits were immunized by a series of four injections of the antigen on alternate days, the amount of the injection increasing from 0.5 cc. on the first day to 2.0 cc. on the last day of the series. Each rabbit was bled from the heart 7–10 days after the last injection. Two subsequent injections of the antigen were made about a month following the bleeding, the first of which was of 0.5 cc., followed 48 hours later by 1.0 cc. All injections were made into the marginal ear vein.

After bleeding, the blood was allowed to clot overnight in the refrigerator. Serum was separated from the clot by rapid centrifugation the following day and was kept frozen until used. The antigen and antiserum were mixed and incubated in a water bath at 37° C. for 1 hour. The mixture was then stored in the refrigerator overnight, centrifuged, and the supernatant fluid injected the following day.

RESULTS

Extracts of chicken caeca, whether from normal animals or from those partially or heavily infected with coccidia, when intravenously injected into chickens, resulted in death. Eleven different extracts, injected into a total of fifty chickens of varying weights and ages, have given similar results. The minimum lethal dose (MLD) was established for some of the extracts. Death was very sudden, usually occurring within 45 seconds. Effects were noticed at 25 seconds and consisted of deeper respiratory movements and defecation, along with violent muscular tremors and stretching and twisting of the neck. Animals decapitated immediately after death did not bleed; and autopsy revealed large clots in the great veins and heart, indicating death due to intravascular coagulation. The other chicken tissues that proved fatal caused a similar type of response. On the other hand, in the two animals injected with pancreas extract, much regurgitation, defecation, and general weakening resulted, but the chickens survived fairly large doses.

Caecal extracts of turkey and guinea fowl proved fatal to chickens; but extract of the lining of the digestive tract of rabbit, guinea pig, and rat produced no visible effect. Comparable doses of chicken caecal extract injected intravenously into rabbits, guinea fowl, and guinea pigs had no effect.

Extracts of lungs of chickens and pigeons were fatal to chickens; the former also killed pigeons and guinea fowls. On the other hand, these extracts in large doses had no effect that could be observed on rabbits or guinea pigs. An extract of rabbit lung was lethal to rabbits, confirming the results of other authors mentioned in the introduction; given in proportionally large doses, it had no visible effect in chickens and guinea pigs. Two chickens, each weighing 1,000 gm., received three injections of rabbit lung intravenously on alternate days. The last injection, consisting of 2 cc., killed one chicken within a minute and the other within 20 minutes. The first and second injections were of 0.5 and 1.0 cc. doses, respectively. A Polish rabbit weighing 1,700 gm. was killed by a 0.25-cc. dose.

It was found that injections of less than the MLD of any of the lethal extracts into chickens produced a refractory period lasting for at least 10 minutes, during which time large doses of any of the lethal extracts could be given without effect. Blood withdrawn from

animals which had received less than the MLD was peculiar in several respects, but this will not be discussed here. Refractory periods after the injection of a tissue extract were first described by Wooldridge (1886).

No systematic study has been made to correlate age or weight with dosage of the extracts. Chickens weighing between 500 and 1,000 gm. were most often used, but fatal results were also produced in adults. The supernatant portion of the homogenized extract was considered as an undiluted solution; and, with caecal extracts, quantities as small as 0.2 cc. killed chickens weighing about 750 gm. In the same-sized chickens, lung extracts of both pigeon and chicken, diluted with three parts of saline, caused death in doses as small as 0.1 cc. One-tenth of a cubic centimeter of undiluted chicken brain and testis extracts yielded similar results.

Since the extracts of tissues that were fatal to chickens produced no noticeable effect in rabbits, with the dosage used, it was possible to give rabbits a series of injections of the extracts. The details of the injection procedures were described above, under "Methods." The sera of immunized rabbits were incubated with the tissue extracts. The usual method was to incubate 1 volume of serum with 1 volume of extract, but in several cases one-tenth and one-fourth the amount of serum was used. The results secured are recorded in Table 2. Complete protection was always secured when equal volumes of the tissue extract and immune serum were used. When the proportions were less than 1 volume of immune serum to 4 volumes of extract, inhibition was not always complete. Tissue extracts incubated with normal rabbit serum in equal proportions occasionally made the test animals very sick without actually killing them. This inhibition appeared to be due to diluting effects; for 1.8 per cent saline alone, when incubated with tissue extract, lessened the toxicity. The inhibition may also have been due to an adsorption of thromboplastin to the serum proteins, as suggested by Mellanby (1909).

TABLE 2

EFFECTS OF TISSUE EXTRACTS WHEN INCUBATED WITH NORMAL RABBIT AND CHICKEN SERA AND WITH IMMUNE SERA PRODUCED IN THE RABBIT

Tissue Extract	Extracts Incubated with—	Results	
		Lethal	Non-lethal
Chicken caecum	Normal rabbit serum	16	7†
	Normal chicken serum	5	0
	Anti-chicken serum	3	0
	Anti-chicken caeca	1‡	22
Chicken lung	Normal rabbit serum	4	0
	Anti-chicken caeca	1§	4
	Anti-chicken caeca lung*	0	10
	Anti-chicken lung	0	2
	Anti-chicken serum	1	2
Chicken brain	Anti-chicken brain	1‖	1
Pigeon lung	Anti-chicken caecum	1	0
	Anti-chicken lung	0	2
	Anti-chicken caeca lung*	1¶	1

* In the fourth injection series this rabbit received both caecum and lung extract. The first three series were caecum alone.

† Diluting effect not taken into account.

‡ Extract and antisubstance mixed just before injection.

§ Very small chicken—250 gm.

‖ Incubated with a much smaller amount of antiserum than the one that protected.

¶ Very concentrated lung extract used. Equivalent to about three times the strength of the other extracts tabulated here.

The immune serum was easily produced against chicken caecum in five different rabbits, against lung in two, and against brain in one; for, after one series of injections of the tissue extract, the rabbit's blood had the power to inhibit the action of the extracts that

caused death in fowls when given alone. Additional injections series increased the potency of the antisubstance. The anticaecal rabbit serum prevented the action of the chicken lung, thus showing a lack of organ specificity. Antisera against chicken caecum, absorbed completely with chicken serum, still prevented the activity of the caecal extract. Antisera against chicken serum had no inhibitory effect.

The lethal effect of the extracts was also inhibited by heparin. In an attempt to find out more about the active principle, caecal extracts were heated for 5 minutes at 50°, 60°, and 80° C. The lowest temperature had no effect, and the highest completely destroyed the activity of the substance. Temperature of 60° C. for 5 minutes gave inconclusive results, for half of the animals tested lived and half died. Filtration through a Seitz filter removed the activity entirely. One sample of caecal extract was lyophilized and lost its potency. These results bear repeating.

In order to study the possible relationship between the pathology of coccidial infection and the thromboplastic effects of the tissue extracts, some preliminary experiments were run. Caecal contents were taken from normal chickens and suspended in a volume of 1.8 saline equal to the volume of caecal contents. These were homogenized and centrifuged, and the supernatant fluid was injected intravenously into chickens. Caecal core material from heavily infected chickens, suspended in saline, homogenized and centrifuged as above, and injected intravenously, killed the chickens with typical intravascular coagulation; but contents from normal chickens had no *in vivo* coagulating effect. Furthermore, an antiserum against the caecal tissue extract apparently inhibited the action of the core material. Too few animals were used on this phase of the work, and the experiments are being repeated.

DISCUSSION

There is little doubt that the fatalities resulting from intravenous injection of the various toxic extracts were due to some clotting factor, such as thrombin, thromboplastin (also called "thrombokinase" by Morawitz, 1904), or tissue fibrinogen (Mills and Matthews, 1924). According to Mills (1927), either thrombin or tissue fibrinogen injected intravenously can cause intravascular clotting. Since Quick (1942) has asserted that Mills's tissue fibrinogen is a mixture of thromboplastin from tissue and of prothrombin from blood plasma, present in the organs extracted, the choice of the active ingredient in the materials we injected is narrowed to thrombin or thromboplastin. Since the source of thrombin is from plasma rather than from tissues, it seems improbable that there would be enough plasma remaining in the tissues after washing with saline to yield sufficient thrombin to cause the massive clotting in our experiments. The lasting potency of wet preparations would also indicate that the effects are probably not due to thrombin, which loses activity rapidly on standing in solution (Mills and Matthews, 1924). Inhibition of the reaction *in vivo* following the injection of heparin reveals little concerning the identity of the substance injected, since heparin may inhibit the action of thrombin (thrombase) and mask the action of thromboplastin.

The fact that results similar to those obtained with chicken mucosa injected into chickens have not been obtained with mammalian mucosa injected into mammals may mean that the chicken mucosa is rich in the clotting factor

and/or that the chicken is very sensitive to the clotting factor. This would also imply that the chicken spleen, liver, and kidney lack any clotting factor detectable by the techniques used.

We have produced in the blood of foreign species substances that, when incubated with the tissue extract, inhibit action *in vivo* of the homologous tissue extract. There are indications that the substance will also inhibit extracts of other organs of the same species. We have referred to this blood as "immune serum"; but, at present, we have no actual proof that the reaction was a typical antigen-antibody reaction. The ease with which the inhibitory substances were built up warrants caution in terming the substance an antibody, in the immunological meaning of the term. There have been previous evidences of antibodies against clotting factors. Bordet, Renaux, and Bordet (1927) demonstrated by *in vitro* methods the serological specificity of the cytozyme secured from stroma of guinea pig, sheep, and goat blood; but they failed to find a species difference in the thromboplastic activity. Cohen and Chargaff (1940) developed antibodies to the protein of a thromboplastic lipoprotein and found that the precipitate resulting from this antigen-antibody combination was even more active in clotting than the antigen alone. They did not describe any experiments with the supernatant fluid. Since they only injected the precipitate, their results are not comparable to those reported here. Mellanby (1933) could find no antibody or resistance against thrombin (thrombase) developed in rabbit after a series of intravenous injections. Tocantins (1940) described an antiprothrombin, and various authors have noted evidences of species-specificity of thromboplastins. We have barely touched upon the problem of the serological specificity of the tissue extracts as correlated with their clotting actions, but such studies are now being undertaken.

The similarity in clotting ability of necrosin, menstrual toxin, caecal cores of chickens infected with coccidia, and tissue extracts, and the fact that cross-immunity tests between necrosin and menstrual toxin and between extracts of caecal core material and the caecal mucosa are possible, may indicate that the breakdown products of most tissues, whether as a result of an irritation or of a mechanical tissue destruction, contain similar physiological substances but with different specificities. The reaction itself is apparently dependent upon the test animal, its sensitivity, and the species from which the material has been secured. With the little knowledge that we have of the purity of the substances discussed, it seems that only broad generalizations are possible.

SUMMARY

1. Saline extracts of chicken caecum and other parts of the digestive tract, brain, and lung are lethal to chickens when injected intravenously. Guinea fowl and turkey caeca and pigeon-lung extracts are also lethal. Death results from intravascular coagulation.

2. Saline extracts of chicken spleen, liver, muscle, and kidney produce no untoward effects in chickens.

3. Proportionally larger doses of the extracts lethal to chickens are tolerated by rabbits. Chicken-lung extract is fatal to the pigeon and the guinea fowl.

4. Extracts of rabbit lung in very small doses are lethal to the rabbit, but large doses are tolerated by the chicken.

5. There apparently is, therefore, a

group similarity in the thromboplastins of the tissue extracts.

6. Immune sera produced in rabbits by injections of chicken caeca, lung, and brain extracts prevent the *in vivo* thromboplastic effects of these extracts.

LITERATURE CITED

BAKER, A. B.; NORAN, H. H.; and LARSON, W. P. 1945. Clotting of blood *in vivo* by aqueous homologous lung extract: some clinical implications. Proc. Soc. Exper. Biol. and Med., **59**:196–97.

BORDET, J.; RENAUX, E.; and BORDET, P. 1927. La Spécificité zoologique des lipoides et spécialement du cytozyme dans la coagulation du sang. Compt. rend. Soc. biol., **96**:141–44.

BOYD, G. L. 1923. The etiology of acute intestinal intoxication in infants. Arch. Int. Med., **31**:297–301.

COHEN, S. S., and CHARGAFF, E. 1940. Studies on the chemistry of blood coagulation. IX. The thromboplastic protein from lungs. Jour. Biol. Chem., **136**:243–56.

LYTSCHKOWSKY and ROUGENTZOFF. 1913. De la toxicité des extraits de proumons d'animaux normaux. Compt. rend. Soc. biol., **75**:45–47.

MELLANBY, J. 1909. The coagulation of the blood. Jour. Physiol., **38**:28–112.

———. 1933. Thrombase—its preparation and properties. Proc. Roy. Soc., London, B, **113**:93–106.

MENKIN, V. 1943. Studies on the isolation of the factor responsible for tissue injury in inflammation. Science, **97**:165–67.

MILLS, C. A. 1921. Chemical nature of tissue coagulins. Jour. Biol. Chem., **46**:135–65.

———. 1927. The clotting properties of pure blood and of pure plasma. Chinese Jour. Physiol., **1**: 249–62.

MILLS, C. A., and MATTHEWS, A. P. 1924. Les deux mécanismes physiologiques de la coagulation du sang. Arch. internat. de physiol., **24**:73–103.

MORAWITZ, P. 1904. Beiträge zur Kenntnis der Blutgerinnung. Beitr. z. chem. Phys. u. Path., **5**:133–41.

QUICK, A. J. 1942. The hemorrhagic diseases and the physiology of hemostasis. Springfield, Ill.: Charles C. Thomas Co.

SCHLOSS, O. M. 1920–21. A substance toxic to guinea pigs in the blood of infants with "intestinal intoxication." Proc. Soc. Exper. Biol. and Med., **18**:101–2.

SMITH, N. R. 1919. Organ extracts. Jour. Lab. and Clin. Med., **4**:517–54.

SMITH, O. W., and SMITH, G. VAN S. 1940. Menstrual discharge of women. I. Its toxicity in rats. Proc. Soc. Exper. Biol. and Med., **44**:100–104.

———. 1945. Evidence that menstrual toxin and "necrosin" are identical. *Ibid.*, **59**:116–19.

TOCANTINS, L. M. 1940. Experimental hypoprothrombinemia with anti-prothrombin serum. Proc. Soc. Exper. Biol. and Med., **43**:729–31.

WOOLDRIDGE, L. C. 1886. Über intravasculäre Gerinnungen. Arch. f. Anat. u. Physiol., pp. 397–99.

INNATE AND ACQUIRED BEHAVIOR AFFECTING THE AGGREGATION OF FISHES[1]

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THE aggregating tendency of many fishes is a well-marked phenomenon. In the extreme form—the fish school, in which all individuals are similarly orientated, uniformly spaced, and moving at a uniform pace—this tendency represents the most closely knit and constant grouping to be found in the vertebrates. Fishes which show this extreme

[1] The experimental work was carried on in the Department of Animal Behavior of the American Museum of Natural History, through the kindness of the chairman, Dr. Frank Beach. He read and gave useful criticism to the manuscript, especially covering the discussion. We are grateful to Miss Priscilla Rasquin for undertaking the necessary optical operations; to Miss Janet Roemhild for technical assistance in preparing some of the apparatus involving color matching and for permission to use certain data incident to her work on another problem; to Lieutenant James W. Atz, who assisted in some of the original preliminary tests made at the old New York Aquarium and later criticized the manuscript; and to Mr. Bernard Nathanson, who assisted in certain mathematical operations.

form of social organization are notoriously difficult to establish under laboratory conditions. This difficulty is not encountered in many other forms which show less rigid groupings. These, while exhibiting strong tendencies to aggregate, form true schools only under special conditions, and then as transient phenomena. Prominent in this category are many species of the Cyprinidae. Their strong aggregating tendencies but limited schooling behavior present certain advantages and are useful in an attempt to inquire further into the mechanism of aggregation and the factors controlling such behavior.

In order to comprehend the purely mechanical aspects of school formation a consideration of the receptor systems involved is necessary. All investigators have found that vision is essential to form or keep a fish school intact. Even in the case of young bullheads, *Ameiurus melas* (Rafinesque), a species with a relatively poorly developed optical system, vision is the primary means of school formation, according to Bowen (1931, 1932). Other species that have been shown to require light and vision for the formation of schools are herring, *Clupea harengus* Linnaeus, by Newman (1876); mackerel, *Pneumatophorus grex* (Mitchill), by Parr (1927) and Shlaifer (1942); dwarf herring, *Jenkinsia stolifera* (Jordan and Gilbert), by Breder (1929); mullet, *Mugil* sp., and golden rudd, *Scardinius* sp., by Boulenger (1929); sea perch, *Morone labrax* Linnaeus, by Spooner (1931); sunfish, *Lepomis auritus* (Linnaeus), by Breder and Nigrelli (1935); goldfish, *Carassius auratus* (Linnaeus), by Breder and Nigrelli (1938); and the characin, *Astyanax mexicanus* (Philippi), by Breder and Rasquin (1943).

Frisch (1938, 1941*a*, *b*) and Goz (1941) have studied the influence of the chemical senses in the aggregating behavior of *Phoxinus laevis* Agassiz, *Ameiurus nebulosus* (Le Sueur), and *Alburnus bipunctatus* (Bloch) and have shown some positive effects. Evidently some substance is secreted which is attractive to their fellows. The work of Breder and Rasquin (1943) suggests a possibly similar condition in the blind cave fish, *Anoptichthys*. While such effects and probably others, such as lateral-line stimulation, may be involved in the maintenance of aggregations, it is, of course, ineffectual in the formation of schools, as here understood in its restricted sense. It is evident from the studies mentioned above that, when light falls to an intensity at which vision becomes impossible, schooling ceases. In all cases tested, this has been found to hold, as, indeed, it must if based on the maintenance of a visual pattern. The very young bullheads should be able to keep together on a tactile basis by virtue of their projecting barbels and thus form a contact aggregation. However this may be, they usually hug the bottom at night and show little if any progressional movement in darkness during their short period of juvenile schooling.

Since the typical fish school is a well-marked phenomenon, it would seem that an accurate definition could be easily framed. However, that is not the case, nor is it usually possible to define the basic nature of the school or even an aggregation, as has been indicated by Deegener (1918), Allee (1931, 1935, 1938), and Breder and Nigrelli (1935). As already noted, the school may be loosely considered as a special form of aggregation in which all individuals are orientated in a common direction, regularly spaced, and moving at a uniform speed.

Several simple problems basic to an understanding of either schooling or ag-

gregating behavior have not been investigated previously. These include the following questions: (1) Does isolation of an individual for a protracted period of time affect its attitude toward aggregating, and is this constant throughout life? (2) Does the presence of phenotypically different color variants affect the formation of aggregations? (3) Does the color of the background against which a fish views its fellows influence the formation of aggregations? (4) Do varying physical and chemical quantities in the water affect the nature of these aggregations? (5) Is it possible to change the nature of a fish's attitude toward aggregation by a system of training? (6) What effect does the presence of blind individuals have on an aggregation? It is to the solution of these various, but clearly related, problems that the present contribution is addressed.

EXPERIMENTAL PROCEDURES

Because of the close approximation to a true schooling condition that an aggregation of *Brachydanio rerio* (Hamilton-Buchanan) will take on under certain conditions, this species was used principally in attempting to ascertain the effects of varying periods of isolation on such reactions. For the studies on the effects of differently appearing specimens on the fish aggregation, *Carassius auratus* (Linnaeus) was employed. This species was chosen because of the possibility of obtaining specimens of vastly different coloration from the same stock and also because certain of these colors closely match those of various aquatic environments, while others are conspicuous under any normal environment that can be easily imagined. In both cases specimens not in breeding condition were used, in order to avoid the complications of sex activity. While the principal problems were divided as above indicated, various cross-references were made between the two species as seemed desirable. The following descriptive parts of the work have been divided according to the two species for purposes of convenience. The discussion considers the basic behavior patterns of both in reference to general concepts.

Brachydanio rerio

It early became evident that even in a large aquarium or outdoor pool the apparent *Brachydanio* school actually was mostly an aggregation in the simple sense of the word. Thus it is evident that this condition is not entirely the result of the early turning-back caused by the never distant glass sides of smaller aquaria. The individual angled darting, typical of the species, also mars what otherwise might be considered a school in the strict sense.

REACTIONS TO ENVIRONMENTAL STIMULI

Perhaps the quickest way to cause this species to form a school is to place a group of them in a new aquarium. Both the fright from handling and the strangeness of the new environment are evidently responsible for the resulting schooling form of aggregation—the crowding-together of panic. This phase generally passes in a day or so or less. After that time the fish tend to herd together less and to distribute themselves more uniformly throughout the tank. Following this, temporary schools of greater or lesser duration may be formed as a result of a large variety of stimuli. Apparently anything tending to startle the fish will cause this drawing-together. The following stimuli were tried; all were successful in causing school formation: (1) sudden movements of large objects outside of the aquarium, (2) sudden jar

of table on which tank stood, (3) sudden increase in illumination, (4) sudden decrease in illumination, (5) rapid flickering light, and (6) sudden extraordinary activity on the part of an individual fish. The effect of all these showed a rapid diminution in a few minutes, the stimulus, after a time, eliciting no evident response or at most a weak one. After a sufficient period of time had elapsed to permit the fishes to become unaccustomed to the stimulus, it again became effective.

VARIATION OF CHEMICAL AND PHYSICAL QUANTITIES

In order to test the effects of variations in the chemical and physical characteristics of water, two identical aquaria, 21.5 by 8.75 by 5.25 inches, were placed side by side, with an equal number of fish in each (19). To establish whether the fish saw and responded to each other through the two glass walls and the intervening air space, a card was at first placed between the two aquaria. As this was moved so as to expose about one-third of the tank, the fish quickly gathered and aggregated as one cluster with the glass partition splitting its middle. Moving the card so as to place the exposure at the other end caused the group to form at that end of the tank. With the card forming a complete separation, the two groups would tend to settle at the center of their respective tanks. This is the position taken by fish aggregations under uniform environmental conditions and in the absence of fluctuating exteroceptive stimulation. An analysis of the reasons for this, in *Carassius auratus* at least, are given by Breder and Nigrelli (1938). This method proved to be convenient for checking any change in social attitude when the chemical or physical nature of the water in one aquarium was varied from that in the other.

These included varying such quantities as the following. The CO_2 concentration was brought near the suffocation level by bubbling CO_2 through the water of one tank. Air was bubbled through the control tank in a similar manner. Sodium chloride was added to one tank up to a concentration of 1.86 gm/l. After a concentration of 0.93 gm/l was reached, the fishes showed a much paler phase (i.e., the chromatophores contracted), but their social attitude displayed no evident change. This occupied a period of 7 days. After a return to normal water for 8 days, their ordinary dark coloration returned, so that they were indistinguishable from the controls. Calcium carbonate was introduced to saturation. The pH rose from 7.3 to 7.5. The physical reactions were similar to those under the influence of sodium chloride. Calcium sulphate was introduced to 0.62 gm/l. The pH fell from 7.3 to 6.9. The physical reactions were similar to those given above. Aquarium "conditioned" water was used in the control and filtered tap water in the other aquarium. In none of these cases did there develop any detectable social difference between the two aquaria.

It thus becomes evident that wide variations in some of the ordinary chemical conditions to be expected under natural conditions induce such slight differences in social behavior, if any, that we were unable to detect them by such methods of observation.

Variations of temperature of from 20.5° to 12.0° C. likewise had no clear effect on the attitude of these fish toward their fellows. The effects of light have already been mentioned and will be further discussed in another connection.

Following the methods of Eddy (1925),

caffeine and urethane were added to two matched aquaria of 6 specimens each. Two concentrations were employed, 0.4 mg. and 0.8 mg. of each in 40 liters. Both showed an effect, the heavier concentration much stronger and more rapid. It appeared that there was a slightly greater tendency toward aggregation in the caffeine aquarium and a lessened one in the urethane aquarium, which is in accord with Eddy's findings on *Ameiurus*. However, this was not of sufficient distinction to be of any service in an attempt to illuminate the experiments in hand. The most noticeable effects involved greater activity on the part of the caffeine group, and probably this was principally responsible for the appearance of greater aggregation.

These conditions could not be maintained for long, for the following reasons. If sufficient amounts were administered to the water to induce the above effects, they also affected the feeding habits of the fishes in a peculiar fashion. Those subdued with urethane, along with their increased sluggishness, showed a lack of attention to food and after a short time began to exhibit starvation effects. Those stimulated with caffeine, while maintaining their appetites, apparently used up more energy than their food could supply and also showed starvation effects. This occurred with greater speed than in the case of the physiologically subdued fishes.

Although this species is adaptable and will live and breed under a wide variety of conditions, it is interesting to note that a mild stimulation will quickly place them beyond the physiological possibilities of continued survival and that a mild subduing of their normal physiological rates will produce a similar effect. Heat and cold, outside of their somewhat restricted temperature range, produce not dissimilar effects.

TRAINING TO SCHOOL FORMATION

Since all the foregoing procedures failed to induce other than a transient school or had other defects that adversely affected their utility for experimental purposes, the possibility of training specimens to form and remain in schools was considered. To this end an apparatus was arranged to provide a punishment every time the fishes became sufficiently separated, in the tentative expectation that they would be able to make the association that they received an "unpleasant" sensation when sufficiently remote from a fellow-fish. Obviously, this could be accomplished only when no more than two fish were present.

The apparatus employed consisted of a small aquarium with two iron electrodes immersed at diagonal corners. These were connected to an inductorium set to deliver just enough current to cause the fish to give a slight twitch when the switch was closed. This circuit was closed and then opened immediately by means of a telegraph key when the fish were as remote from each other as the aquarium permitted—a matter of about 12 inches.

Under these conditions it took approximately one-half hour for about forty opportunities to present themselves. By this time it was clear that the punishment of the mild electric shock was causing an association to be established. The association made by the fishes at this time was definite and distinct but not at all of the nature anticipated. At this time the reaction was for either or both fish to rush at the other and to bite at its fellow. These fish lack jaw teeth and are evidently unable to inflict any considerable injury. They can exhaust one another, however, by such attacks. Thus by the time about fifty shocks had been administered, one of the fish was rather badly beaten and doubt-

less would have died if the experiment had been continued.

Twenty-four hours later, both fish seemed to be perfectly normal again. The experiment was resumed, and on the sixth shock the attacks began again and ran a similar course. Like results were obtained with a second set of two other specimens.

Evidently in such an arrangement with a fellow-fish, the association of shock is referred to the only other motile object present, but in such a way as to prevent the possibility of training the fishes in this manner, since the association established is "antisocial" rather than "social." It is, however, of interest in another connection, as it gives an indication that these ordinarily peaceful and aggregating fishes evidently can refer "unpleasant" experiences to their companions. So far as we know, this is the first time this phenomenon has been shown in fishes. Among mammals such results are not uncommon in experiments employing more than one animal in which use is made of some form of punishment, frequently electric shock, for the "wrong" acts. Dr. Frank Beach assures us that it is the case with many species.

As all these various attempts failed to induce other than a transient fright school, they suggest that the *Brachydanio* social aggregation is rather stable in constitution and not subject to distinct modification by either environmental "pressures" or ordinary learning processes, at least those involving punishment for motivation.

NUMBER OF INDIVIDUALS INVOLVED

Because each individual fish may be considered as possessing a sphere of influence about it, it follows that a larger school, within limits, should be more cohesive than a group of a few fishes. Expressed another way, a small group should be much more easily subject to scattering than a larger group. To check this, various groups of fishes (from 2 to 19) were separately examined. It was evident that the larger the group, the fewer were the stray fish to be found away from the main body.

COLOR OF BACKGROUND

Since such schools are visual aggregations, it follows that the visibility of a neighboring fish should be expected to have some influence on the strength of social attitude. In order to test this, a tank 3 feet by 1 foot by 6 inches was employed. Opaque materials were used to avoid side-light and silhouette effects. This tank was painted flat black on the inside. Two fish were placed in the tank and their relative positions noted every 10 seconds for one hundred times, being counted as aggregating when less than the length of a fish apart. The readings from this series of experiments are given in Table 1, *A*. Although it will be noted that these readings are quite irregular, it must be remembered that there is an inherent weighting away from "aggregation," because when the two strung out in a line they were counted as not aggregating, whereas in a larger group they certainly would have been still in the aggregation.

Experiment 1 was performed with 2 males and the rest with females. The difference between the sexes shown in Table 1 suggests that the males may be more attentive to their fellows than are the females; but, of course, much further work would have to be undertaken to determine this point. All readings taken together show the fish to be aggregating 42 per cent of the time. This is with but 2 fish in the black-painted tank.

Later, 12 specimens were introduced, and, although it was then impossible to

make the same kind of observation, it was clearly apparent that a much more definite school was formed. It was estimated that 7 out of the 12 were in the aggregation most of the time, while 5 were ranging about. All these experiments with the black tank terminated by the fish hiding in one of the corners. Aggregation, however, could usually be produced by some disturbance, such as an increase of light. The same fish when transferred to an ordinary glass aquarium aggregated immediately and maintained this for some time, usually the larger part of one day.

The black tank was then painted white for half of its length. Ten fish were introduced, and their relative positions in regard to background are given in Table 1, *B*. The observations show a considerable tendency to remain on the dark background, although the background seemed to have no effect on their aggregational behavior. The fish happened to be less conspicuous on the background on which they aggregated.

TABLE 1

SOCIAL ATTITUDES OF *Brachydanio rerio* UNDER VARIOUS CONDITIONS

A. TWO FISH IN A BLACK-PAINTED TANK

Each aggregation figure represents the number of times together out of 100 observations at 10-second intervals. Two different fish were used in each experiment.

Expt. No.	Per Cent of Observations in Aggregation	Mean Value
1	84, 42	63
2	12, 1, 7, 19, 0, 0, 11	7
3	87, 90, 17, 6	17
4	0, 13	6
Entire series		42

B. TEN FISH IN A TANK HALF-BLACK AND HALF-WHITE

Each aggregation figure represents the number of fish on white out of 100 observations at 10-second intervals. Observations made on 3 consecutive days.

Day	Per Cent of Fish on White	Mean Value
First	0, 1, 2, 2, 5, 3, 3, 0, 5	2.3
Second	3, 0, 0, 0, 0, 0, 0	0.4
Third	0, 0, 0, 0, 0, 0, 0, 0, 0	0.0
Entire series		0.9

C. EFFECTS OF TARGETS MOVED PAST AN AQUARIUM

(Based on over one hundred trials)

Size of Card (In Inches)	Reaction
2×2 and smaller	Always positive
2×8	Positive to weak negative
4×8	Positive to weak negative (about neutral)
8×8	Always negative

ARTIFICIAL FISHES AND THEIR EFFECTS

A simple, string-operated device was arranged that could cause an artificial "fish" or other object to "swim" past, just outside the glass tank wall, arranged so that it could be observed from a distance by the operator, without disturbance. A small white or black card roughly the size and outline of a *Brachydanio* caused the specimens to congregate near and at times attempt to swim along parallel with it in a manner apparently similar to "schooling" with companion fishes. Like other reactions of these fishes, after a few attempts no further responses could be elicited from the subjects until half an hour or so had passed, when the cycle of attraction could be repeated.

After this had been established, simple rectangular cards of various dimensions and areas were substituted, and they also elicited responses. The effects of the various-sized cards are given in Table 1, *C*. Consistent results were obtained with

two different sets of fishes, set up in different places. In all, something over one hundred trials were involved. It was evident that a moving area of over 32 square inches caused retreat, while smaller sizes tended to cause advance, down to 4 square inches or less, where reactions were always positive. The effect is, however, complicated by the following items: (1) The fish quickly lose interest in a positive target, just as they do in any individual fellow-fish they may have been following; and (2) when a negative target passes out of sight they tend to converge to where it *was*, although avoiding it when visible. These two effects, coupled with the rapidity with which they become accustomed to a given stimulus, makes the use of such a method somewhat difficult.

BEHAVIOR OF VARIOUSLY ISOLATED INDIVIDUALS

Newly deposited eggs of *Brachydanio* were isolated, one to an aquarium, while still in their early cleavages.[2] The aquaria were provided with separating opaque paper-board "blinders," so that it was impossible for any fish to see another in an adjacent aquarium. A peculiar feature developed, in that it was found exceedingly difficult to rear many of these fish, although there was no difficulty encountered with the rest of the eggs not so segregated but in identical aquaria. Unless there was some obscure difficulty, not evident but inherent in the aquaria used—such as a slight toxicity which several eggs could overcome but which would be fatal to a solitary egg—it would appear that the survival rate is very low among such eggs when isolated. This would seem to be most probably an extension of the growth stimulation which homotypically conditioned water induces in many such fishes, a matter discussed by Allee (1935) and noted by Bowen (1932). Out of 70 eggs so established, only 2 produced viable young, and one of these died as a fairly small fish. One grew to full size and then, at the age of about 6 months, began to show what was apparently some sort of muscular dystrophy or possibly a kind of kyphosis of the caudal peduncle. This fish, which was, nevertheless, vigorous and in good color, was then transferred at 6 months and 1 week of age to an aquarium of about 15 similarly sized fish that were showing a fair degree of aggregation. The disturbance incident to introducing the new fish led to an immediate formation of a school. The introduced fish, which up to that time had never before seen any fish, immediately schooled with them with no hesitancy whatever. This was a vigorous reaction on the part of the introduced fish. The group, naturally, retreated to the far end of the aquarium on the introduction of the net, and the released fish swam straight toward and into the school. Because of its slightly crippled condition, it was at all times completely recognizable. With this physical handicap it could neither swim so fast nor was it so agile as the others, but it nevertheless showed perfectly co-ordinated schooling reactions. Another experiment of this sort again produced a single, but apparently fully normal, fish, which showed identical behavior and soon became "lost" because it could not be distinguished from the others. Evidently aggregating in this species is not in the nature of a learned reaction but has its genesis in some innate mechanism. This seemingly simple fact has

[2] As the eggs of this species are demersal and nonadhesive, there is no difficulty in handling them. They were placed individually in conditioned water in aquaria which measured 10 by 8 by 6 inches in 5.5 liters.

considerable bearing on other experiments.

The crippled fish, described above, on joining the school, caused considerable activity on the part of other individuals. This is frequently the case when a new fish is introduced into an aquarium. Probably partly because of this but more especially because of the different swimming motion that this fish showed, it became the center of considerable attention. This culminated in others actually butting it with their heads. Furthermore, such activity was noted off and on for a month or more.

It is to be noted that the very young fry of this species do not aggregate to any notable extent; but after about 2 months of age, when they are already of essentially adult form, they begin to show this reaction on fright. Evidently this reaction does not appear until some certain level of physical development is attained. Prior to the development of this reaction, fright causes scattering in all directions; but after its development the drawing-together is very marked. If a group does become divided into two by a sufficiently violent action on the part of a net, the groups will rejoin even if it involves the necessity of individuals passing close to the object causing the fright. Generally the smaller group will rejoin the larger.

Adult fishes that have been kept in isolation for a period show quite other reactions on rejoining a group. All so isolated for 6 months at an age of 6 months failed to rejoin a school immediately. In one case it took nearly a week for a merging to occur, although most specimens rejoined in about 1 day. During this period the newly introduced fish kept to that part of the tank most remote from the aggregation, and this entailed finding a different depth level, either above or below that used by the aggregation. Thus, the innate reaction to aggregation may evidently be modified by experience after it has once been exercised and then abandoned because of enforced isolation. Unlike many species, *Brachydanio* retains its aggregational behavior throughout life, which runs to as much as at least 3 years in aquaria. At best a senile failure to aggregate precedes death by only a few days at most and probably should be looked upon as a premoribund condition. Further experiments with such isolated Brachydanios are considered along with the *Carassius* experiments, since they involve other techniques which are therein discussed and with which they are strictly comparable. These data are given in Table 5. Since this species is fully adult before 6 months of age under favorable conditions, there is no evident basis for supposing that the difference in age of fish 6 months old and 12 months old has any bearing on the foregoing observation.

Carassius auratus

Before it is possible to determine whether or not fish distinguish between companions of varying color, it is necessary to determine if such fishes are differentially influenced by the color of the background against which they appear. For such a study the common goldfish is peculiarly well suited. The "normal" wild goldfish is a slaty, olive-gray, very similar to the color of a carp and very inconspicuous over a wide variety of pond bottoms. The reddish-yellow genetic variant (the ordinary goldfish of commerce), differing in a loss of melanic pigment and an intensification of the erythrophores, is notably conspicuous, on the other hand, over practically any pond bottom. Both types, as well as variously variegated specimens, may be obtained

from a single spawning of a suitable pair of goldfish. The color varieties are thus intimately related; and, unless there is some gene-linkage connecting behavior with color variation, these fishes would be expected to show similar behavior, no matter what their color—unless there is some unlikely innate or learned behavior differential, involving some sort of self-recognition concerned with color.

Compared with other species, goldfish show remarkably little adjustment of color associated with background, the gray ones darkening or lightening slightly according to the background, and the yellow ones to an even less degree. It has been shown by Sumner (1934, 1935*a*, *b*) that *Gambusia*, which has somewhat more ability to lighten or darken on appropriate backgrounds, becomes restless on a background to which it has yet to adjust itself. His bibliography mentions numerous similar cases in other forms. Brown (1936) and Brown and Thompson (1937) discuss similar items, and the latter show that various species of Cyprinidae tend to choose a background more nearly matching their own color phase. Many species go far beyond this, showing extensive color and pattern change, as well as choice of background, as is seen, for example, in the flounders, as demonstrated by Mast (1916). This is also true of other groups, as, for example, in the insect *Corixia*, as shown by Popham (1942, 1944). Breder (1946) gives an extended discussion on the possible role of this feature of fish behavior. Sumner (1944, 1945) discusses the modification of certain physiological processes in response to the visual appearance of the environment.

REACTIONS TO MATCHING BACKGROUNDS

With this kind of material and this general background of fish behavior, the following experiments were undertaken. A trough 39 by 48 inches was painted, one-half yellow and one-half gray, so that there were two adjacent areas of each color 39 by 24 inches. The depth of water was 2 inches, and the sides of the trough were painted similarly, carrying the corresponding colors of the bottom upward. The two colors were carefully prepared to match the two colors of the fishes used, as far as was practicable. It was obvious at once that the fish were outstandingly conspicuous on the alternate color and decidedly inconspicuous on the matched color, at least to the human eye. Since there is no reason to suppose that goldfish are lacking in color vision (Walls, 1942), it is presumed that they received somewhat similar retinal color values. Even if it were subsequently shown that this particular species is unique among teleosts in lacking color vision and that they saw only shades of gray, there was still a marked difference between the two surfaces in this respect, the yellow end of the trough being much the brighter.

The daylight of the laboratory was supplemented by overhead light and was sufficiently uniform to be within the limits, established by Shaw, Escobar, and Baldwin (1938), between which locomotor activity is practically unaffected by light intensity. The temperature of the water was held between 24° and 29° C., within which range activity is large. In this connection it is important to recall that memory in goldfish has been shown by French (1942) to be influenced by changes in temperature, the interpretation of which has been criticized by Jones (1945). The temperatures used by French ranged from 4° to 28° C., which are so extreme for over the short periods of time employed that shock effects should be expected. In the present work the temperatures were not allowed to

vary more than 5°. Boulenger (1929) stated that various yellow fish, including *Carassius*, were more active at night, a feature which was certainly not true of the present specimens or any other goldfish known to the authors. Szymanski (1914) showed by experimental means that goldfish activity lessens on low light intensity, as did Shaw, Escobar, and Baldwin (1938) and Spencer (1939).

Four fish, either all yellow or all gray, were placed in this trough and their positions noted in periods as given in Table 2. The methods employed were those of Breder and Rasquin (1943). That is, the number of specimens in either half of the trough were noted at 5-second intervals; and any value under 100 indicates that the fishes spent most of the time in the gray background, and any value over 100 indicates that they spent more time on the yellow, 0 and 200 indicating, respectively, entire avoidance of one color, which is to say that the values indicated represent percentage of random expectation. It is obvious that in Experiments 1 and 2, both grays and yellows showed closely similar behavior, indicating a very considerable avoidance of the yellow end. Each experiment was divided into two equal parts, in which the colors were switched end for end to overcome any possible bias inherent in the physical aspects of the trough. The significance of these results is perhaps more clearly indicated in Figure 1, which gives a graphic representation of the data and indicates the unanimity of reactions of the two differently colored sets of fishes.

TABLE 2

EXPERIMENTS ON GOLDFISH PRESENTED WITH TWO COLORS OF BACKGROUND

In this notation 100 represents indifference or random expectation, and the values are in percentage as departures from that value.

Expt. No.	Fish	No. of Observations	Percentage of Random Expectation		
			Maximum	Mean	Minimum
1.....	4 yellow	1,000	89.5	59.2	31.5
2.....	4 gray	1,000	105.0	58.4	0.0
3*.....	4 yellow	500	121.0	103.5	69.0

* During this experiment a part of the yellow paint peeled and stood out as a vertical rectangle of about 2 by 3 inches and exposed an equal dark undersurface.

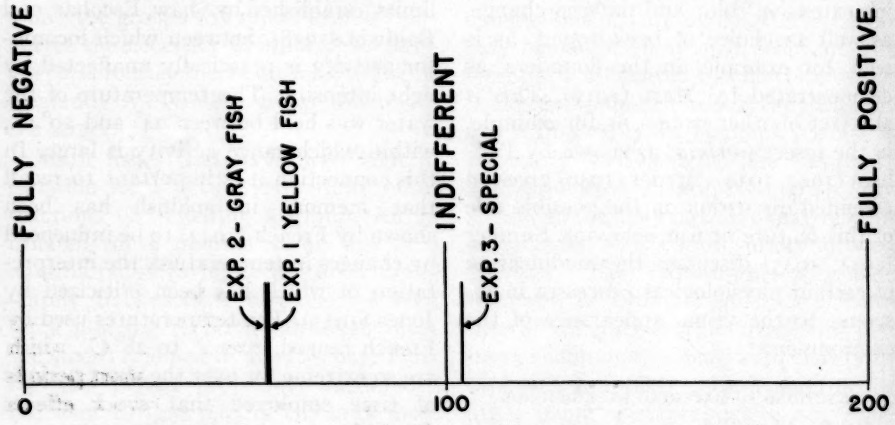

FIG. 1.—Chart of mean values from Table 2. Scale expressed in terms of attitude toward a yellow background, in which indifference is indicated by 100 and fully positive by 200. See text for full explanation.

Experiment 3 followed in time Experiments 1*a* and 2*a*. Here there appeared a slight peeling of some of the paint, so that a small rectangle of yellow paint raised from the bottom and stood vertical, reaching nearly to the surface. This presented a new element in the environment and, aside from its companion fishes, was the only object that a given fish could see. This clearly attracted the fish and just about neutralized the repellant effect of the yellow, as is indicated in Table 2 and Figure 1. This defect was corrected, and Experiments 1*b* and 2*b* gave readings similar to those of the first set.

The tendency of the fish was to "hide" on the gray side. Both gray and yellow fish could frequently be seen to swim up to the line of demarcation and then back off, stopping short of swimming over onto the yellow background. The gray fish were thus by this item of behavior more or less confined to a background against which they were decidedly inconspicuous; but the yellow fish, behaving in the same way, maintained themselves in the most conspicuous manner possible.

If the distribution of the various possible groups of 4 fish is counted, the essential similarity between the behavior of the two colors of fishes appears in another way. This is clearly shown in Figure 2, *A*. The differences between the slope of the curve of the grays and that of the yellows is clearly too slight to have any evident significance. The experiment with the upraised piece of paint (Expt. 3) shows a quite different form, again indicating the essential neutralizing effect of the total of conditions in this experiment. It approaches the form of the curve of simple binomial distribution, the sum of the influences nearly canceling each other so as to give the appearance of randomness. Compared with the curves obtained by Breder and Rasquin (1943) on blind characins, which showed almost pure random distribution, this serves to indicate how slight a modification of a "blank" environment, if balancing the previous condition, will distort a strong bias to a single factor toward what appears to be a theoretical randomness.

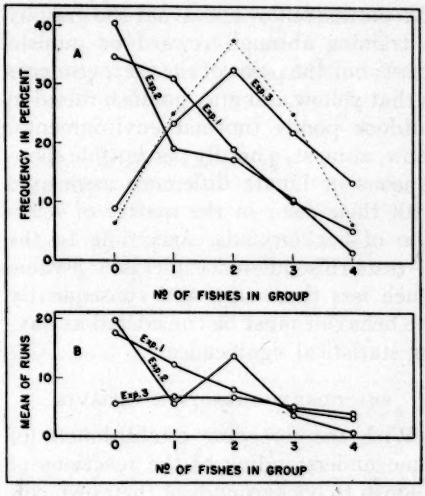

FIG. 2.—Distribution of 4 fishes according to grouping in terms of percentage of occurrence on yellow half of trough. *A*, Referred to binomial distribution in which the theoretical expectancy is shown as a dotted line. *B*, Expressed in terms of mean of longest "run" of consecutive numbers in group.

If the longest consecutive sequences of any one number of associated fishes are tabulated, the same general situation is apparent, as is indicated in Figure 2, *B*. All the long sequences of Experiments 1 and 2 are on gray and slope off rapidly to small values of the larger aggregations on yellow, while these data for Experiment 3 are nearly a straight horizontal line, as would be expected because of the neutralizing effect of the peeled paint.

It was found that there was not any

significant drift as the experiments moved through their successive periods of observation, which were separated by half-hour intervals. In other words, in a period covering $2\frac{1}{2}$ hours, in which 500 observations were made, divided into five units, these fishes did not show any recognizable change in their attitude toward a yellow background. There is little doubt that they could be trained to live on the yellow and avoid the gray by a training through reward or punishment; but the point of these experiments is that yellow and gray goldfish raised in outdoor ponds (normal environments) show, at most, a hardly perceptible spontaneous or innate difference associated with their color in the matter of selection of backgrounds. According to the χ^2 test, this difference yields a p-value much less than 0.05, and consequently the behavior must be considered as having statistical significance.

REACTIONS TO PHENOTYPIC VARIANTS

With the foregoing establishment of some understanding of the reactions of goldfish to backgrounds of their own color, it is now possible to obtain some measure of their attitude toward individuals of their own color or of different colors in connection with the backgrounds against which the fishes are living. For this purpose the same trough was employed, but it was divided into two parts, so that there were now two troughs, one completely yellow and the other gray, on both bottom and sides. Each measured 39 by 24 inches. These were both divided optically into two equal parts by means of a thread stretched across the middle above the surface of the water. Into these compartments all the possible combinations of the two color types of fishes were studied by similar methods of noting positions at either end of the trough. A tabulation of these combinations is listed in Table 3, *A*, by experiment numbers. The distribution of the fishes given in terms of size of aggregation is also given in this table. The general aspects of these data may be more clearly expressed graphically, as is done in Figure 3. From the data of Table 3, *A*, and Figure 3, it is evident that goldfish show a different aggregating tendency when one of them is of an odd color.

Also, it is clear from Figure 3 that the alternate conditions as listed in Table 3 show close resemblances. It is to be noted that *A* and *B* of Figure 3, which represent experiments of one color of fish, all show a characteristic U-shape. *F* and *G*, which represent the behavior when there are three similarly colored fish and one odd colored, do not show this U-shape but present a distinctly different form. When the two sets of data are combined, as in *C* and *H*, respectively, this difference becomes all the more marked. The four curves going to make up *C*, as already noted, are convex downward. They approach closely to the reciprocal of binomial distribution, a feature which will be discussed in another connection. On the other hand, the four curves going to make up *H* show no such marked regularity and, when combined, show a high value for three. The details and significance of this will be taken up at another place, since it is sufficient to indicate here that 4 fish of similar color and 4 fish, one of which is different from the rest, show vastly different aggregation configurations. This is reinforced by the data of Breder and Rasquin (1943), which forms the bases of *E*, in which they used 5 yellow fish, and indicates its approach to the reciprocal of binomial distribution. *J*, which shows the data based on experiments involving 4 fish, 2 of which are gray and 2 yellow, again shows the U-

shaped form and graphically is indistinguishable from the single-colored aggregations. There is, however, a subtle difference here, in that evidently these groups acted nearly as two separate groups of 2 each, combining to form a similar figure but on a different basis.

The experiments shown in Figure 3 indicate the fish against a yellow background by a solid line and those against a gray by a dashed line. A critical examination indicates that the background makes for no statistical or evident difference between their behavior. However, when these experiments are examined in reference to whether an odd-colored fish matches or contrasts with the background, certain tendencies appear to be expressing themselves. Thus in *A* and *B* there is more resemblance between gray fish on gray and yellow fish on yellow and again between gray fish on yellow and yellow fish on gray than between the two matched experiments. It is evident that fish on a matching background stayed closer together than

TABLE 3

DISTRIBUTION OF VARIOUS COLOR COMBINATIONS OF GOLDFISHES IN REFERENCE TO BACKGROUNDS AND COMPANIONS

Expt. No.	Background Color	Gray Fish	Yellow Fish	Percentage of Fish in Each Possible Aggregation				
				0	1	2	3	4
	A. Before Isolation							
4	Gray	1	3	9.7	29.2	22.6	23.9	14.6
5	Yellow	3	1	16.3	16.0	19.2	35.7	12.8
6	Gray	3	1	33.5	16.3	10.2	32.2	7.8
7	Yellow	1	3	25.5	18.9	13.8	33.4	8.4
8	Gray	.....	4	31.4	10.5	10.8	15.4	31.9
9	Yellow	4	.....	29.1	16.0	13.7	13.6	27.6
10	Yellow	.....	4	42.8	17.7	5.3	6.5	27.7
11	Gray	4	.....	45.5	5.7	7.0	7.8	34.0
12	Gray	2	2	34.3	6.6	7.0	10.5	41.6
13	Yellow	2	2	44.2	13.1	5.5	6.9	30.3
	B. After Isolation							
14	Gray	4	.....	25.4	5.2	8.9	21.7	38.8
15	Yellow	.....	4	3.3	43.6	18.9	8.3	25.9
16	Gray	3	1	35.6	11.4	6.6	13.5	32.9
17	Yellow	3	1	7.2	17.8	16.5	32.4	26.1
	C. With Blind Companions							
18	Gray	3	1 blind	20.2	20.7	15.1	23.9	20.1
19	Yellow	3	1 blind	10.3	17.1	22.8	29.1	20.7
20	Gray	2	2 blind	13.1	25.0	18.5	21.7	20.7
21	Yellow	.....	2+2 blind	16.0	26.6	16.7	20.4	15.3
22	Yellow	2	2 blind	10.8	20.7	25.9	40.2	12.3
23	Gray	.....	2+2 blind	14.0	23.6	25.0	27.8	28.1
24	Gray	.....	4 blind	8.9	28.5	37.2	20.3	5.1

they did on a contrasting background. This probably means little more than that, with lessened contrast and visibility, they did not permit their fellows to wander as far as when they were distinctly visible against a contrasting background. As should be expected on this basis in experiments with 2 fish of each color, as shown in *J*, the configuration is so close as to permit of no clear separation.

It should be borne in mind that these fish were tank-mates at all times prior to and between experiments and were in no case presented with a new element in the environment in the form of a different color of companion or a "new" companion.

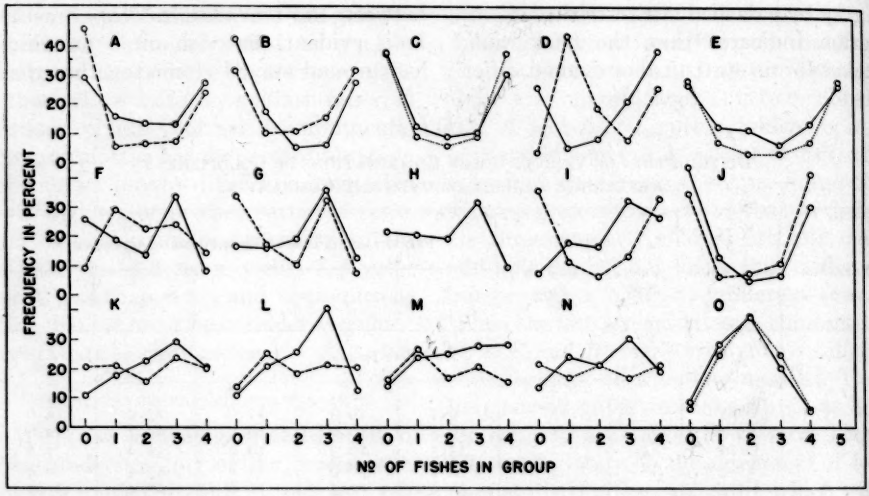

FIG. 3.—Experiments with various combinations of gray, yellow, and blind *Carassius*. *Top row; all fishes of one color: A*, Solid line (Expt. 9), gray fish on yellow background (1,000 obs.); dashed line (Expt. 11), gray fish on gray background (1,000 obs.). *B*, Solid line (Expt. 10), yellow fish on yellow background (1,000 obs.); dashed line (Expt. 8), yellow fish on gray background (1,000 obs.). *C*, Solid line (Expts. 8, 9, 10, 11) (4,000 obs.); dotted line (reciprocal of binomial distribution). *D*, Solid line (Expt. 14), gray fish on gray background after isolation (1,000 obs.); dashed line (Expt. 15), yellow fish on yellow background after isolation (1,000 obs.). *E*, Solid line (5 yellow fish on gray background; data from Breder and Rasquin [1943]) (200 obs.); dotted line (reciprocal of binomial distribution). *Middle row; gray and yellow fish mixed: F*, Solid line (Expt. 7), 1 gray and 3 yellow fish on yellow background (1,000 obs.); dashed line (Expt. 4), 1 gray and 3 yellow fish on gray background (1,000 obs.). *G*, Solid line (Expt. 5), 1 yellow and 3 gray fish on yellow background (1,000 obs.); dashed line (Expt. 6), 1 yellow and 3 gray fish on gray background (1,000 obs.). *H*, Solid line (Expts. 4, 5, 6, 7), 3 similar and 1 odd-colored fish (4,000 obs.). *I*, Solid line (Expt. 17), 1 yellow and 3 gray fish on yellow background, after isolation (1,000 obs.); dashed line (Expt. 16), 1 yellow and 3 gray fish on gray background after isolation (1,000 obs.). *J*, Solid line (Expt. 13), 2 yellow and 2 gray fish on yellow background (1,000 obs.); dashed line (Expt. 12), 2 yellow and 2 gray fish on gray background (1,000 obs.). *Bottom row; blind and visually intact fish: K*, Solid line (Expt. 19), 1 blind yellow and 3 gray normal fish on yellow background (1,000 obs.); dashed line (Expt. 18), 1 blind yellow and 3 gray normal fish on gray background (1,000 obs.). *L*, Solid line (Expt. 22), 2 blind yellow and 2 gray normal fish on yellow background (1,000 obs.); dashed line (Expt. 20), 2 blind yellow and 2 gray normal fish on gray background (1,000 obs.). *M*, Solid line (Expt. 23), 2 blind yellow and 2 normal yellow fish on gray background (1,000 obs.); dashed line (Expt. 21), 2 blind yellow and 2 normal yellow fish on yellow background (1,000 obs.). *N*, Solid line (Expts. 20, 21, 22, 23) (4,000 obs.); dotted line (mean of binomial distribution and its reciprocal). *O*, Solid line (Expt. 24), 4 blind yellow fish on gray background (1,000 obs.); dotted line (binomial distribution).

BEHAVIOR OF VARIOUSLY ISOLATED INDIVIDUALS

After the preceding experiments had been completed, the fish were placed under various kinds of isolation for a period of 6 months. Certain fish were isolated individually, while others were isolated only as to color. The former were used for experiments with fishes of one color and the latter for mixed groups. Table 3, *B*, and Figure 3, *D*, show the results of experiments with fish of one color, each on its matching background, which, as has been indicated, makes for a more cohesive group. The gray fish gave a curve not unlike that obtained before isolation, but the yellow gave one which resembles those of fishes of mixed color. Taken together, it is evident that the behavior has been modified, at least in the yellow fish, by isolation. Those which had been isolated as to color only, shown in *I*, with the odd fish yellow on a yellow background, showed a configuration like those obtained before, while those on gray showed a very cohesive group. Two of these four configurations taken together serve to indicate that, whatever manner of social organization is originally present, it may be considerably disrupted by a period of isolation. It is to be noted in this connection that when these tests were undertaken it became immediately apparent that the fishes behaved differently and were more alert to stimuli arising outside the trough. Although formerly the presence of the observer showed no particular effect, after 6 months of isolation the fish appeared "nervous," and a reflection of this may be seen in Tables 6 and 7 in the notable bias to one end of the trough. A careful examination of the original data failed to reveal any tendency for these fish to drift toward the former pattern during the time it took to make the experiments, although it was at first thought that these experiments might show such a tendency, since it is only in the first period of the first experiment that the fish can be considered as having been isolated, the rest of the time representing the period of accommodation toward the new companions. Evidently it takes a considerably longer time to re-establish such a social organization than the duration of these experiments provided.

THE EFFECT OF BLIND INDIVIDUALS ON THE GROUP

Reference has been made to the close approximation to binomial distribution that is shown by both normally and experimentally blind characins, as demonstrated by Breder and Rasquin (1943). Since it does not necessarily follow that goldfish when blind would show exactly the same pattern, it was necessary to check this item on the species under consideration. Tables 3, *C*, and 4 indicate these data and show that the behavior is essentially the same. That is, when goldfish lose their vision, their movements are no more separable from binomial distribution than are those of the characins, as is shown in Figure 3, *O*. It is of interest in this connection that the optically intact characins do not have the same aggregating pattern as normal goldfish. The former remain quiet, in a compact aggregation, and become active only on some special stimulus, which made it impossible in that study to measure their activity by this method. Thus loss of vision reduces these two species to a common behavior pattern, although they had previously showed marked differences.

Experiments 18–23, inclusive, concern mixed groups of blind and optically intact goldfish. Since the blind fish show a close approach to binomial distribution and the seeing fish a similar close ap-

proach to its reciprocal, it would be expected that a mixed group would show some intermediate. That is seen to be the case, as indicated in Tables 3, *C*, and 4 and Figure 3, *K–N*. Furthermore, it is to be noted that in the mixed groups the values 3 and 1 tend to be high. In those with 1 blind fish and 3 seeing, the failure of the former to aggregate tends to make the occurrence of an aggregation of 3 or 1 fish more frequent. Those with 2 each of blind and seeing produce a similar pattern, but in this case of slightly different genesis. Since there were 2 fish wandering at random and 2 fish tending to aggregate, they tended to group with one or the other of the blind fish, obviously rarely being able to include both. They did not tend to split up into two groups of 1 blind and 1 seeing in each, since between the 2 seeing there was the double action of each affecting the other. Since in Experiments 20–23, inclusive, half the specimens are blind and half seeing, it might be thought that, mixed in equal parts, they would approach the mean of binomial distribution and its reciprocal. Actually, they tend to vary as the reciprocal of this mean value, as is clearly indicated in Figure 3, *N*. The reasons for this are indicated in the preceding paragraph. This gives a measure of the interaction between the blind and the seeing, which is tantamount to saying that if the seeing paid no attention to the sightless, then only could one expect the calculated values for binomial distribution and its reciprocal to be approximated.

TABLE 4

COMPARISON OF AGGREGATIONS UNDER VARIOUS GROUPINGS

Expt. No.	No. of Observations	Aggregations in Per Cent					
		0	1	2	3	4	5
8–11 incl.	4,000	37	12	9	11	30	.....
4– 7 incl.	4,000	21	20	16	32	11	.....
20–23 incl.	4,000	13	24	21	27	19	.....
12–13 incl.	2,000	39	10	6	9	36	.....
14–15 incl.	2,000	14	24	15	15	32	.....
16–17 incl.	2,000	21	15	11	23	29	.....
18–19 incl.	2,000	15	19	19	26	20	.....
24	1,000	9	28	37	20	5	.....
(B. and R.)*	6,184	7	23	36	26	8	.....
(B. and R.)†	200	27	13	12	7	14	27
Frequency of 4 terms:							
1.‡ Binom. dist.	.....	6.25	25.00	37.50	25.00	6.25	.....
2.‡ Recip. of 1	.....	37.50	9.37+	6.25	9.37+	37.50	.....
3.‡ Mean of 1 and 2	.....	21.88−	17.18+	21.88−	17.18+	21.88−	.....
4.‡ Recip. of 3	.....	18.12+	22.81+	18.12+	22.81+	18.12+	.....
Binom. dist. of 5 terms	.....	28.46−	7.69+	3.85−	3.85−	7.69+	28.46 −

* From Breder and Rasquin (1943); distribution of *Anoptichthys* under comparable conditions.

† From Breder and Rasquin (1943); distribution of five yellow *Carassius* under comparable conditions, in a gray soapstone trough.

‡ Refers to numbered expressions in text (p. 174).

COMPARISON WITH *Brachydanio*

Some experiments identical in technique with those performed with goldfish were undertaken with *Brachydanio* for purposes of comparison. It rapidly became apparent that this manner of ap-

proach was not nearly so useful for this species because of the great attention given by *Brachydanio* to outside influences. It should be borne in mind in this connection that the utility of this technique varies with the general behavior of the species involved. Those species most suitable for such studies have at least the following characteristics: (1) continued locomotor activity, (2) slight attention to exterior influences, and (3) slight social modification by immediate history. Eyed *Astyanax* were found by Breder and Rasquin (1943) to be entirely unusable in such experiments for the first reason, and *Brachydanio* approaches the limit by reason of the second and third. Theoretically, these two could be compensated for by a more elaborate apparatus, involving, perhaps, a separate vibrationless building and with the observations made through peep sights.

Nevertheless, the results obtained on the trials made indicate clearly some of the differences inherent in the organization of the *Brachydanio* and *Carassius* aggregations. These differences are in complete agreement with various items implicit in the other types of experiments on *Brachydanio*. These tests were all made in a gray soapstone trough otherwise identical with the goldfish experiments.

Specimens taken from a group of tankmates that had grown up together showed the "binomial reciprocal" common to uniformly colored goldfish. The data are given in Table 5 and Figure 4, *A*. The latter compares with Figure 3, *C*, of the goldfish series.

Adult specimens were individually isolated for a period of 6 months, with the original intention of making experiments comparable to those on similarly isolated goldfish. However, at the end of 6 months, owing to the difficulty in maintaining these fishes in such isolation, it was found that there were only 3 normal survivors and 1 cripple. The latter was the same fish that had originally been reared in isolation and then, after being introduced to an aggregation, had been left with them for a period of about 3 months. These 4 fish were nevertheless tested, with the results shown in Table 5 and Figure 4, *B*. The irregular nature of the curve obtained agrees with that of goldfish similarly treated, as shown in Figure 3, *D*. However, it so happens that data yielded by goldfish of mixed colors also produced similar irregular curves even when not isolated, as is shown in

TABLE 5

DISTRIBUTION OF VARIOUS COMBINATIONS OF *Brachydanio*

Expt. No.	Percentage of Fish in Each Possible Aggregation				
	0	1	2	3	4
1 (4 normal fish recently assembled*)	31.5	12.2	9.0	9.9	37.4
2 (3 normal fish and 1 cripple from individual isolation)	2.9	22.0	3.8	4.9	30.5
3 (4 normal fish, one of which had not been isolated)	49.3	18.3	11.7	7.9	12.8
4 (4 normal fish, after living together one week)	10.3	34.4	27.4	18.8	9.1

FREQUENCY AT ONE END OF TROUGH, AS COMPARED WITH THEORETICAL EXPECTATION

Expectancy for 4 Units	200
Experiment 1	209.6
Experiment 2	253.8
Experiment 3	116.4
Experiment 4	184.1
Mean of all	190.9

* The data of this experiment were taken by Miss Janet Roemhild.

Figure 3, *F–H*. It is thus impossible to distinguish from this experiment which influence is in control or whether both are present. The form of the cripple and its locomotion were different from that of the other 3 and thus introduced a disharmonious element that may have had an effect on the curve obtained not dissimilar to that found in goldfish on a color basis. Other data, not pertinent to present matters and to be reported elsewhere, are confirmatory of this view.

After the initial part of the first experiment, these fish could no longer be considered as isolated. Thus, after a week in isolation, another fish which had been in an aggregation was similarly isolated, and then these 4 were tested, with the results shown in Table 5 and Figure 4, *C*. Here, clearly, a more cohesive group was formed, the curve of which is convex downward, as is to be expected in such aggregating fishes. The large asymmetry of the curve is evidently associated with the ease with which outside influences disturb this species.

Finally, these 4 fish were kept together for a week, that is, "isolated" together instead of being part of a large group, as in the first case in this series of experiments. The results are shown in Table 5 and Figure 4, *D*. This curve is convex upward. Like that of Figure 4, *B*, it is subject to various interpretations. It was influenced, however, by the fact that one of the specimens seemed to be becoming sickened, in that it was sluggish and did not keep up with its companions, which accounts for the high value of aggregation 1. The whole form of the curve may be influenced by the rapid adjustments found in these fishes by other experiments. That is, after a time of exposure to a given set of conditions, the fishes of an aggregation pay progressively less attention to one another. On the other hand, it is possible that some kind of association with handling and transferring from trough to aquarium had been set up and referred to other individuals somewhat comparable to the results obtained in the electric punishment experiments.

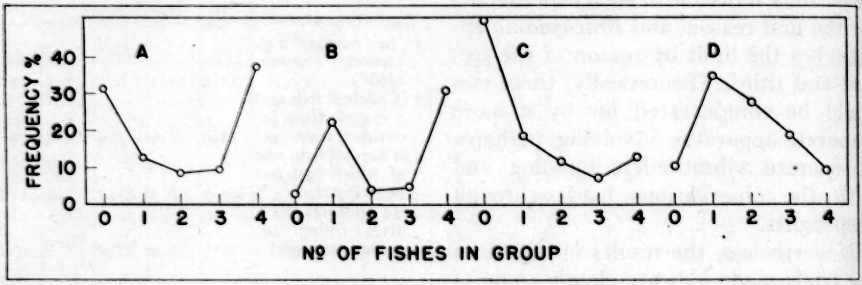

FIG. 4.—Experiments with *Brachydanio rerio* in various conditions and combinations. *A* (Expt. 1), Four normal fish from a larger aggregation (1,000 obs.). *B* (Expt. 2), Three normal and 1 crippled fish that had been individually isolated for 6 months (1,000 obs.). *C* (Expt. 3), Four normal fish, 1 of which had not been isolated (1,000 obs.). *D* (Expt. 4), Four fish of *C* after living together for 1 week (1,000 obs.).

It is clear from this short series of experiments, when taken into consideration with the other experiments on this species, that the attitude of *Brachydanio*, both to its fellows and to other elements in its environment, is considerably different and evidently more complicated than in the case of the comparatively sluggish and more "placid" goldfish. The whole effect would seem to be one of more im-

mediate and overt response to stimuli in the more active, alert, and "nervous" of the two species. This agrees well with general observations and also serves to indicate the differential applicability of this technique.

DISCUSSION

The experiments reported herewith have certain significance bearing on aggregational and schooling behavior in fishes and on the broader subject of general ichthic behavior patterns, which may not be fully obvious. For this reason the following discussion has been extended to include a general review of the literature concerned with social behavior in fishes, so that the perhaps seemingly separate items of the experimental portion of the paper may be viewed in their proper perspective as items in a general patchwork of data that is slowly being fitted together by a variety of investigators. In addition to this, where practicable, reference has been made to what appears to be comparable behavior in other groups of animals. As the subject is naturally somewhat involved, the treatment has been to break the matter up into more or less arbitrary groups of items under subheadings for purposes of clarification; but, as with all such matters dealing with what is essentially a single unit, in which each fragment is inextricably interwoven with the others, such treatment tends to give a false appearance of separateness.

EXPERIMENTAL RESULTS

The following list with its references to pages and tables serves to consolidate the chief points of the experiments. The foregoing experiments indicate that the following features are present as elements in the aggregations of certain fishes of the family Cyprinidae.

1. Relative size of object in motion, whether fishlike or not, determines whether it will cause advance or retreat, but all such stimuli soon lose their effectiveness because of the rapidity with which these fish become accustomed to a given stimulus (p. 160; Table 1, *C*).

2. Fright in any of its numerous aspects will cause aggregating and in its extreme form will cause temporary schools. No other influence that has been brought to bear seems to operate in just this way, although many other noxious influences will cause aggregations in various animals. Nor is training toward this end by punishment effective, since the methods employed lead to fighting (pp. 156 and 158).

3. The formation of aggregations is innate when a certain level of physical development is reached, although later it may be modified by the past history of the individual (pp. 161, 169, and 170; Tables 3, 4, and 5).

4. Brachydanios react positively to a black background and negatively to a white background. *Carassius*, whether gray or yellow, collect on a gray background rather than on a yellow one. A slight tendency for the yellow fish to be less negative than the gray members of that species is noted (pp. 159 and 163; Tables 1, *B*, and 2, 3, and 4).

5. Groups of like phenotypes are more cohesive than mixed groups, the variants evidently being recognized by the others (p. 166; Tables 3, 4, and 5).

6. Normal or experimentally produced blind fish closely approximate binomial distribution in their aggregations, but phenotypically-uniform eyed forms approach the reciprocal of binomial distribution, while mixed seeing and blind fishes form intermediates which approach the reciprocal of the mean of the two (p. 169; Tables 3 and 4).

Table 4 consolidates the data illustrative of various of the preceding points. These values and those obtained by Breder and Rasquin (1943) on *Anoptichthys* pertinent to the present study are compared with binomial distribution. This includes only part of their data, all of which indicate similar behavior. The experiments chosen for representation here were those of a single group of blind fish, directly comparable in number to the present work on goldfish. Blinded goldfish behaved like normally blind cave fish, as is indicated in Tables 3, *C*, and 4; and Figure 3, *K–O*.

Since the data obtained from these experiments are related to binomial distribution by the nature of the methods employed, analysis of the figures may be properly made in reference to the expansion of the usual binomial expression $(p + q)^n$ expanded for each term. The significance of comparing these experimental results with various forms of this expression resides in the fact that the comparison exposes a degree of regularity and relationship in these aspects of aggregation that is similar to simple mathematical concepts. Further work along the lines suggested here should eventually permit the establishment of an adequate measure of how much of animal behavior can be considered in terms of transformations of algebraic equations.

It was found that blind fish alone subscribed to this expression, as shown in Table 4 and Figure 3, *O*. This may be taken to mean that such fishes are little influenced by the activity of their companions and are evidently moving in such a manner as closely to approximate randomness. Thus the first expression may be written as follows:

$$(p + q)^n, \qquad (1)$$

in which p and q each $= 0.5$, the two areas of the trough, and $n = 4$, the number of fishes.

It is also evident that fish which more or less follow each other and form aggregations would not yield values which this expression provides. The values obtained from such aggregating fish would be expected to be high for the *O* and 4 positions and low for the intermediate ones, as graphically shown in Figure 3. These values could take conceivably any form of U-shaped figure. That they do, however, tend to approximate the reciprocal of expression (1), as shown in Figure 3, *C* and *D*, was not anticipated, nor are the biological implications of these particular values clear at this writing. Why the possession of vision would change a random distribution to one of a definite form is clear, but just what "the reciprocal of randomness" indicates in terms of vision or its lack, while interesting as a problem, is considered without the province of this communication. The reciprocal of binomial distribution is perhaps best represented by the following expression:

$$(p + q)^{-n}. \qquad (2)$$

Since expressions (1) and (2) were closely approximated by certain of the experiments and since still others were undertaken with half the number of fish of the type which subscribe to one of the expressions and half to the other, the mean of expressions (1) and (2) were considered as a possible point of reference. The result is a series of fluctuating values expanded from the following expression:

$$\frac{(p + q)^n + (p + q)^{-n}}{2}. \qquad (3)$$

Compared with the values actually obtained from the experiments, it was found that they were high where those of the expression were low and vice versa, as shown in Figure 3, *N*. Thus the values

obtained from the experiments were evidently not far from the reciprocals of expression (3), which becomes

$$\frac{2}{(p+q)^n+(p+q)^{-n}}. \qquad (4)$$

This is evidently a reasonably close approximation to the values actually obtained. Here again the biological implications are not entirely clear, but they would seem to be connected with the fact that the fish with vision are attentive to all companions, including the blind.

The values for the expansions of these four expressions are given in Table 4 for direct comparison with the values obtained from the experiments. At least, we can say at present that the above expressions tend to be approximated by the values obtained from the experiments in the following manner:

Expression (1) is very closely approximated by normally blind *Anoptichthys*, and blinded *Astyanax* and *Carassius*.

Expression (2) is nearly equally closely approximated by visually intact *Carassius* of one phenotype and by *Brachydanio*. The case of visually intact *Astyanax* is a special one, in which the 4 fish are so closely tied by visual contacts that the only distribution that can be obtained is with all 4 together in one or the other areas. Thus the group can be considered only as a unit that can be handled by expression (1), in which $n = 1$ instead of 4, the values of which would be 50, 50 expressed in terms of percentage.

Expression (3) has not been found to be approximated in the experimental results here reported. It does appear under the influence of certain sexual combinations, which will be reported elsewhere. However, its reciprocal, expression (4), has been found to be approached in the present material.

Expression (4) is reasonably approached by combinations of optically intact *Carassius* and blind ones.

Mixtures of differing phenotypes of *Carassius* and various degrees of isolation of *Carassius* and *Brachydanio* produced modifications of these basic tendencies that are not so readily referable to comparison with extensions of the expression $(p + q)^n$. They are, however, reducible to conditions represented by expression (4) if the opposite ends are combined, which cancels any bias set up by incidental conditions. These values appear only after isolation, as in Figure 3, *D* and *I;* in unequal mixed groups, where there is only 1 odd-colored fish and 3 uniform, as in *F*, *G*, and *H;* or in mixtures containing some blind fishes, as in *K*, *L*, *M*, and *N*. The comparatively erratic distributions are evidently characteristic of such mixing. There is some slight evidence that this is not so erratic as it would appear but rather is displaying some regularity, which only further experimentation and analysis could reveal and on which there is not sufficient evidence at this time to warrant further discussion.

Such considerations lead inevitably to others, which involve a consideration of the purely mechanical elements that form part of all organic activity. Before a thorough understanding of activities peculiar to a given organizational level may be obtained, it is necessary to differentiate clearly such activities from those that are common property with various lesser orders of organization, which, in this case, means a clear separation of activities peculiar to the sentient as compared with the nonsentient, a discussion of which is given in the following section.

MECHANICAL ASPECTS OF AGGREGATION

First of all, it should be borne in mind that the tendency to aggregate is not re-

stricted to motile living units. Various aggregates of nonliving origin are commonplace. These differ from aggregations of living units only in so far as any nonliving and living activities differ. The former are acted upon directly, and the latter, in addition, are modified by the intervention of biological processes. In both there must be (1) some material, which by virtue of the total of its properties can be made to aggregate; (2) an appropriate moving force or activation; and (3) some particular conditions of the environment that determine the nature of the aggregate. Examples are abundant; a few are given in Table 6, including nonliving and both nonmotile and motile living items listed under the items numbered above.

In the first two examples, organic activity does not enter even remotely; yet in the second not only do the iron filings aggregate, but they orientate and all "point" in an appropriate direction, which is one of the features of the fish school in the restricted sense. The next three are of organic origin, yet all but the last might just as well be of inorganic origin. *Lemna* sinks in the fall because of its increased weight due to the accumulation of starch grains. From there on, the activity might just as well involve nonliving items. When the *Lemna* has sunk, the wind-induced currents cause the separate plants to move along the bottom, entangling one another so that they become rolled up into balls of various sizes, the aggregation being not unlike that of a snowball rolled along the ground.

Long lists of aggregating animals might be made in which condition 1 is the species and the condition of the individuals aggregating, 2 is almost always the animal's own locomotor efforts, but 3 is highly variable, such as the seeking of food, physical comfort, sex, and so on. Deegener (1918) attempted such a classification, based on what elements hold an animal aggregation together, and

TABLE 6

EXAMPLES OF AGGREGATION INVOLVING NONMOTILE AND MOTILE OBJECTS

(1) Objects Acted Upon	(2) Moving Force or Activation*	(3) Condition of Environment (Internal and External)	(4) Results
	Nonmotile Objects		
Pebbles	Water current and flow	Shape of stream bed	Piles
Iron filings	Magnetic force	Shape of magnetic field	Clusters
Leaves	Wind	Obstruction causing eddy	Piles and windrows
Nuts	Gravity only	Shape of bowl	Pile
Lemna	Wind-induced water currents	Accumulation of starch	Ball-like clusters
	Motile Objects		
Mackerel	Locomotion (swimming)	Social drive (retinal demand?)	Schools
Starlings	Locomotion (flight)	Social drive (retinal demand and warmth?)	Flocks
Ants	Locomotion (crawling)	Social drive (chemical demand?)	Colonies

* In each of these, gravity is considered as understood, only the added influence being mentioned.

found it necessary to make a greatly extended list, although his is probably the barest outline of what urges animals to group together. An extended discussion of the nature of such classifications is given by Allee (1931, 1935).

As previously indicated, this is as much as to say that in inanimate objects aggregation or dispersion is due to the direct action of physical forces on suitable material, while in individuated items (biological units) it is intermediated through the receptor-effector system. This is merely a statement of the difference between sentient and nonsentient. Aggregations to be intermediated through the nervous system presuppose some locomotor ability. When that is absent, the aggregations must be of the physically direct level. Probably oyster aggregations approach such an origin, where the spat presumably settles on everything, only those surviving which come to rest on suitable objects.

Further examples of animal activity can be classified, such as schools of mackerel, flocks of starlings in the fall, droves of ants, etc., as is indicated in Table 6. Thus, when the drives happen to include fellow-items, we have the genesis of an aggregation, school, flock, or social organization, evidently held together by a multitude of biological drives.

It is important to remember that the original simple physical "force and form" concept is never escaped, and it is necessary to be careful to distinguish between those elements which are intermediated through the effector-receptor system and those which are not, before any adequate analysis may be made. Living items simply change the face of the activity picture. For example, a group of dead mackerel may be swirled into an aggregation by current alone, but live ones aggregate because of their responsiveness to a certain visual pattern. Orientation is likewise not separable in terms of sentient responses. Fish in a school will all head the same way. Fish in a stream flow will do the same without any necessary reference to each other, for purely mechanical reasons. Thus by studying only the more complex activities of animate items it is easy to confuse the basic physical features with them. Even the peculiar milling behavior of fish (Parr, 1927) and ants (Schneirla, 1944) is also represented in the purely physical: an eddy of whirling leaves, a water spout, a whirlpool, or any tangential force about some formation that translates into a closed figure.

Complicating the picture in these general terms, there is a negative type of aggregation which results largely from the inaction of dispersal activity. Such a type is often seen in clusters of plants whose seeds have not fallen far from the parent-stock. In fact, the interaction of these two influences—dispersion and aggregation—covers the whole gamut of population structure.

All the foregoing also applies to fish aggregations of the nonschooling type. Because of the density of water, aquatic animals, if they are to move at any considerable speed, must necessarily be somewhat streamlined. Thus it follows that fishes move with speed correlated to their extent of streamlining. Consequently, given a number of such fishes, all impelled by some common impulse or tropism to go to some one place simultaneously, they must of necessity somewhat take on the appearance of a school. Conversely, if they are in a flow of water and attempt to hold their position, then again they must face in one direction, into the flow, and swim upstream as fast as the moving water carries them down. May it then be said that schooling is merely a mechanical result of stream flow induced by a fish moving forward or in a current? Since positions in currents are held by aid

of the eyes, as has been shown by Lyon (1905, 1909*a*, *b*), may it be that the school is held together simply because one fish happens to take another as a point of reference instead of a rock or other stationary object? While undoubtedly this element enters the problem to a large extent, it is not so neatly disposed of as such a view might suggest. This may be stated in spite of the fact that the present experimental data have shown three of the features of fish aggregations to be evidently approximating simple mathematical expressions, which certainly are suggestive of a decidedly mechanistic influence. But why should another fish be taken as a point of reference? Is it because it displays a motion and is consequently more noticeable? Why do not lagging members of a school drop out to station themselves whenever a jutting rock happens to catch their eye? These questions, while more difficult of approach than those which have been studied, would seem amenable to experimentation.

After the receptor system has passed on its data and these emerge again as an effective act, the fish swims to the appropriate place it is to take. The details of this have been worked out by Parr (1927). He interprets the integrating activity to be of two parts: a positive tropistic attraction between fish and fish and a tendency to swim in parallel courses. He sustains the thesis of "a simple automatic 'attraction' towards a perceived companion" as the only necessary basis for the schooling behavior of fishes.

Failure of this "attraction" to operate would seem to be due to various inhibiting influences, a thesis further developed by Parr (1931) and Breder and Nigrelli (1935). The present experiments bear on this in that they call attention to the relatively basic changes in aggregations that may be induced by modifying various features of companionship. A consideration of these features leads us away from the purely mechanical aspects and into the biological influences, which are not too well understood. The question of why there should be this strong mutual attraction is still undeveloped, but the factors that are involved in the operation of this phenomenon may be primarily divided into external and internal.

A consideration of the crude imitations that will elicit social reactions is rather striking, and the literature contains many references to items of this nature that include a wide variety of animals other than fishes. When it is borne in mind, for example, that Breder (1936*a*) could elicit at will spawning or fighting reactions in a male sunfish by merely moving a piece of oval gray cardboard more or less after the fashion of a female and male, respectively, and that Shlaifer (1941) could induce tarpon to rise to the surface to breathe in imitation of a model going through roughly similar motions, it is not surprising that *Brachydanio* would attempt to school with a bit of rectangular cardboard if of a reasonably appropriate size.

In connection with these results it is interesting to note that Rand (1941), in studying the reactions of young thrashers, *Toxostoma curvirostre*, found that size was of importance in determining whether a bird moved toward or away from an object and was able to write: "The tendency to move from a larger and toward a smaller object was indicated in the experiments with the cylinders, the cylinders resembling no natural object."

BEHAVIOR PATTERNS AND AGGREGATION

Since behavior patterns are built up on a basis of both innate and learned reactions, it should not be surprising that

such a complex social reaction as aggregation should display evidences of its basis in both kinds of reaction. Fishes run the entire gamut of attitude on aggregation from the basically solitary to the highly organized schooling type, and, further, these attitudes frequently change in the development of the fish from one type of attitude to another. Without going into specific details at this time, it may be said that the more solitary types of fishes have their social behavior marked by such activities as hiding from or fleeing from others, fighting, holding territory, and feeding without the presence of other fish, while strongly aggregating forms are marked by such activities as seeking others, lack of fighting, the holding of no specific territory, and hesitancy about feeding in isolation. These further develop, at least sometimes, pecking cycles and orders of dominance. Although the normally solitary forms may do likewise under conditions of captivity, such activity frequently leads to the destruction of those on the lower parts of the scale, and sometimes all but a single dominant specimen may be destroyed. All these items, as well as others to be discussed subsequently, have direct bearing on the nature of the aggregation. For example, the present studies have shown that the influence of isolation alone will vary with the age of the individual involved, so that in *Brachydanio*, at least, aggregating is evidently an innate reaction but is subject to subsequent modification conditioned by the recent history of the individual.

The work of Scott (1942, 1944) on the social organization of mice is interesting in present connections chiefly in that he found that these animals, which live in family groups and hold territory, do not show any evident imitative behavior. On the other hand, the same investigator, Scott (1945), in studying sheep, found the imitative factor very large and important to the social organization of the flock. While it would be difficult to work out experimentally, his findings lead to the expectation that if these two mammals could be treated as the fish have been, the mice would approximate the expression $(p + q)^n$ and the sheep $(p + q)^{-n}$. Parr (1931) has already sufficiently discussed similarities and differences between schooling fish and herding ungulates.

EFFECTS OF AGGREGATION ON ORGANIC ACTIVITY

It has been shown that groups of fishes have certain very definite effects on the physiology of the individuals, and there are probably many more that could be determined. It has been adequately demonstrated that fishes consume more oxygen when alone and when crowded than when in groups of intermediate size. Bowen (1932) worked with *Ameiurus melas* (Rafinesque), while Schuett (1933) worked with *Carassius auratus*. Their results are in essential agreement, and evidently the increased oxygen consumption is associated with greater locomotor activity. Schuett (1934) and Escobar, Minahan, and Shaw (1936) demonstrated this increase in the activity of solitary fish, and Shlaifer (1938, 1939) demonstrated the association of the two factors and reconciled apparent conflicts in the earlier work. Breder and Nigrelli (1938) also confirmed this increase in locomotor activity and further showed that it partook of a different pattern when a fish was alone than when it had companions. Their values for spatial positions in three dimensions, while not directly comparable to the present data, greatly resemble the present approximation of a binomial and its reciprocal for a fish with companions and when alone, respectively. While this might seem to be the reverse

of the present interpretations, actually the lone fish with its persistent travel about the confines of the aquarium is comparable to the uniform groups, and the group which centers in the midpoint of the tank is comparable to the blind fish. On the one hand, the measure is of actual spatial position of one fish and, on the other, the frequency of distribution of a group. If an eyed fish and a blind one were so measured separately, this apparent reversal would disappear.

Schuett (1934) and Escobar, Minahan, and Shaw (1936) further indicated that the goldfish responds both to crowding and to small populations by swimming faster than when it is one of some intermediate (optimum?) number. Breder and Nigrelli (1938) observed that the change in spatial adjustments is clearly referable to the attractiveness of fish to fish, and the difference in behavior is a measure of the strength of that tropism. Working with *Tarpon atlanticus*, Shlaifer and Breder (1940), Shlaifer (1941), and Breder (1942) showed in this form, in which aerial respiration is necessary, that the periodic rises to the surface for air are modified by the presence of companions, as evidently imitative efforts come into play. Visual contact with more active forms, such as *Carassius*, increases both the locomotor activity of *Tarpon* and its rises for respiration.

The attitude of strongly schooling fish to handling is notably different from that of nonschoolers. The former are generally so sensitive to handling that they are seldom seen in captivity (Breder, 1936). The semischoolers are less difficult, and the solitary fishes generally still less so.

ENVIRONMENT AND AGGREGATION

While it is impossible to discuss aggregational behavior without reference to the environment and the foregoing parts of the discussion contain references to details which equally well belong here, it should help orientation to examine these and others according to the elements which go to make up the totality of the environment. These may be primarily divided into physical, chemical, and biological.

Of the physical elements in the environment, light is of prime importance, since true schooling is fully dependent on vision, and, except for little-known and evidently comparatively feeble effects due to olfaction and lateral-line senses, so, too, are simple aggregations. This was indicated in the introduction, where there are given a large number of references.

Temperature, since it modifies the behavior of fishes, is also intimately involved in their social attitudes. Thermal changes may induce schooling, which consideration made it necessary to hold the temperatures constant in the present work. The sunfish, *Lepomis auritus* (Linnaeus), when subjected to temperature of less than 7° C., will form a school, but only if a current is present. Breder and Nigrelli (1935) found not only that a current was necessary but that its rate of flow and direction determined closely the form that the school was to take; in fact, they could mold the school into practically any form by regulating these two quantities. From this they concluded that the positions sought were those requiring a minimum of muscular activity in response to the low temperatures. Integration of the school was caused by the fishes using each other as points of reference in a flow, as previously discussed. For these reasons the present studies were carried out in standing water. A worth-while extension of these studies would be to carry out similar experiments under various conditions of flow. As is evident from the above, flow of

water is also inextricably involved in the formation of schools and aggregations.

Chemical influences have been distinguished as sometimes having a pronounced effect on aggregating tendencies. Breder and Nigrelli (1935) were able to induce aggregation in sunfish under special conditions by suddenly increasing the CO_2 content of the water.

In the case of the sunfish cited above, still water with a CO_2 concentration of 0.17 mM/l induced school formation. These experiments were carried on subsequent to the thermal ones, and it is thought that the school formation was due to associative memory of the previously flow-formed school—at least, Shelford and Allee (1914) have shown that reactions to dissolved gases are partly referable to rapidly adjusted responses on the part of the fishes. The speed of development of such adjustment and the establishment of conditioned reflexes in fishes are generally rapid and must be continually guarded against in such studies. Aestivating cichlids, when subjected to a rise in CO_2 concentration of over 0.90 mM/l, cease fighting and tend to aggregate, as shown by Breder (1934). The aquarium in which these latter experiments were made was one of stationary water. If a flow had been induced in it, a school would have been necessarily formed for mechanical reasons.

It has been shown by Keys (1930) that oxygen consumption varies with its concentration, an effect that seems to be operative only in the extreme ranges.

The influence of biologically "conditioned" water on social attitudes, if any, in the case of fishes is not clear. Shlaifer (1938) found no difference in "group effect" in such water, although it has been abundantly shown by Allee (1935) and others that such water has effects on growth, reproduction rate, and longevity in various animals.

It has been shown by Eddy (1925) that certain stimulants cause aggregation and certain depressants cause dispersal in young *Ameiurus*. However, this entire subject on the effect of the chemical environment of the aggregating habits of fishes needs further study. The whole question may conceivably be reduced to the relative effects of depressants and stimulants on the receptor-effector systems, which interpretation would lead to Eddy's observed results. However, in the case of the present experiments, such effects led to other complications, which may be due merely to what appear to be very limited tolerances on the part of *Brachydanio* to the substances used.

The data herein reported are mainly concerned with other individuals considered as elements in the environment of a fish. These may include large predators whose appearance can induce the formation of a school in aggregating fishes.

Fright may cause momentary disruption of a school (Parr, 1927), as in the case of a rock dropped into such a group. This would seem to be little more than the avoidance of too close an approach to a large solid object—the sudden scattering disrupting the serried ranks—and would seem to be a more violent form of what Breder (1929) showed for small herring moving in dense schools about a wharf. These avoided close approach to solid objects and consequently left vacuities about each, the two branches of the school reuniting on the other side, with the frequent formation of complicated whorls as a result of what may be considered as a "stream flow."

On the other hand, feebly schooling fishes can frequently be caused to form schools by a sudden fright, as has been shown herein for *Brachydanio*. A similar effect of the passing of a shadow has been

shown by Spooner (1931) for the sea perch, *Morone labrax* Linnaeus, and has been seen in many fishes in aquaria, e.g., pilotfish, *Seriola zonata* (Mitchill); yellow jack, *Caranx chrysos* (Mitchill); and bitterling, *Rhodeus amarus* (Bloch). It is probably in this class of phenomena that the calling behavior of brooding cichlids belongs, as described by Breder (1934) for *Aequidens latifrons* (Steindachner) and by Noble and Curtis (1939) for *Hemichromis bimaculatus* Gill. As the juvenile school advances and tends to become more loosely integrated, the parents can condense the young quickly by swimming over them and waving their fins in such a fashion as to cause rapidly moving shadows. This the parents regularly do when they detect what may be danger, by virtue of their greater visual range and past conditioning. Finer-drawn effects, such as those involving phenotypic variants, as is shown herein, may be demonstrated to have a profound effect on the behavior of the group and consequently on the social organization.

When one considers environment in broad terms, fishes may be thought of as existing in two main types: the littoral, when solid objects are present as part of the familiar surroundings, and the pelagic, when there are no fixed points of reference. It is perhaps not remarkable that in the latter is to be found the fullest development of the schooling habit.

AGGREGATION CYCLES

As fishes are strongly influenced in their behavior by all the rhythmic environmental cycles to which they are exposed, it follows that this is impressed on their social organization. Although all familiar with fishes in a free state are aware of this, there is surprisingly little solid data of a sort usable in present connections. While solitary species are usually to be found on the territory they are holding, the daily rounds of activities undertaken by schooling fishes are not nearly so clear. A small school of *Strongylura ardeola* (Cuvier and Valenciennes) studied by Breder (1932), which contained an abnormally colored individual and was thereby recognizable, would appear at regular intervals off a dock, always coming in from a certain direction and heading off in another definite bearing, as though the fishes were moving over a more or less fixed circuit. At the same place a large school of *Lutianus griseus* (Linnaeus) swarmed under the same dock during the day but was absent at night. Examination of stomach contents showed that they contained organisms that could be obtained only from a coral reef some half-mile away. This whole problem of the daily rhythm of behavior in schooling fishes invites study.

The longer seasonal cycles modify the social behavior markedly. The seasonal temperature change alone is a potent influence, and, of course, the sexual cycle causes perhaps the most prominent differences in the attitudes of individuals. Migrations became prominent in many forms, and here again the effects of the migratory impulse on aggregation and vice versa invite study.

POPULATION DENSITY AND AGGREGATION

One of the most prominent effects of the typical school is on the population density. This is, of course, true of any form of aggregation, but the effect is undoubtedly directly conditioned by the length of time the population spends in a condensed condition. It has been shown that the experimental populations of the guppy, *Lebistes reticulatus* Peters, reach stable values arrived at from either above or below (Breder and Coates, 1932).

Considered in a broad way, all the

mackerel in the ocean represent a very thin population; but, because of their schooling tendencies, mackerel always live in exceedingly dense populations; in fact, there is only "swimming room" between them. Since visual stimulation of miscellaneous sorts is exceedingly slight in pelagic environments, there is relatively little for such fish to see except each other, minute food objects, and occasionally large enemies. Since it has been so well established that "floating" fish require some visual point of reference to determine their whereabouts, it is not surprising that oceanic fishes are generally given to schooling. With few exceptions it is only in such relatively "blank" environments that the fish school reaches its ultimate development. The difficulty of disruption of such aggregations is more or less proportional to the amount and kind of other objects in the visual field. This may be demonstrated on *Fundulus* in large tanks with and without various kinds of fittings.

The formation of the school, among other items, is in part a function of the efficiency of the receptor-effector system in its ability to co-ordinate appropriately its activities to its sensory impressions. A comparison of maze learning between solitary and schooling species of fishes should be illuminating in this connection. It would seem likely on a priori grounds that fishes dependent on others as objects of response might learn mazes in a very different fashion from fishes always "on their own." The latter, by the nature of the case, would certainly seem to be habitually paying more attention to inert objects in their environment. Welty (1934) has already undertaken some experiments with goldfish and showed that they learned slightly faster in groups. He found that this was not true in the case of the mud-minnow, *Umbra*, and referred the difference to mechanical interference, conditioned by the nature of his experiments. The latter's comparatively solitary disposition may have had an influence as well. Differently devised experiments on truly solitary fish would help arrive at a fuller understanding of the extent of social facilitation in both types.

AGGREGATIONS IN ONTOGENY AND PHYLOGENY

The internal factors bearing on the formation of the fish school may be considered from a number of standpoints. From that of ontogeny it is clear that the developmental stage in which a fish happens to be determines very largely its attitude toward school formation. The vast majority of species aggregate or school as larvae, post-larvae, or juveniles and then lose this attribute with more or less speed as maturity is reached. These juvenile schools are frequently formed as soon as the fish are able to swim and sometimes are exceedingly mixed in character, not only containing various species and sizes but presenting a most heterogeneous appearance. In mature fish a solitary life of a few months is sufficient to inhibit the aggregating impulse, as shown herein. Breder and Nigrelli (1935) expressed the view: "Schooling may be considered as a primary impulse, with cases of non-schooling as inhibitions of it, caused by feeding, reproduction or other requirements. Any cessation of such influences causes an immediate reappearance of the schooling habit."

Considered in a phylogenetic sense, the fishes which display schooling most prominently are in well-demarked groups. The herrings, the mackerels, and the jacks are possibly the most outstanding. Of these, only the herrings have other than oceanic representatives. Among these are the most striking fresh-water schooling fishes. Appropriate response to other objects is frequently completely absent in

oceanic schooling fishes. Consequently, it is sometimes impossible to keep them in captivity unless a circular school or mill is formed, for otherwise they will crash into tank walls, rocks, or other objects and so shortly destroy themselves (Breder, 1936*b*).

In the light of these considerations concerning aggregating tendencies as exhibited by fishes, the results of the present studies may now be considered. It has been shown that the behavior of yellow goldfish with regard to background is essentially like that of the original gray-colored ones, although, in a state of nature, such behavior would certainly be of negative survival value. The very slightly lessened tendency of the yellow fish to avoid a yellow background may be evidence of a slight drift in the direction of more suitable behavior in the matter of matching a background.

If we consider the history of the yellow variant of the goldfish, the question might be raised as to why they have not developed some compensatory behavior. Originating in China many hundreds of years ago, the variety has a long lineage. It is a commonplace that goldfish released in natural waters revert in comparatively few generations to the "wild" gray type. This is generally believed to result from the conspicuous nature of the yellow ones, which leads to their rapid elimination by predators. If there are any other factors, such as a lesser viability of the yellow variant, it has so far escaped notice. Behavior under adverse circumstances does not suggest any greater hardihood of the gray fish as compared with the yellow. On the other hand, certain "fancy" goldfish, such as fantails, telescopes, lion-heads, and so on, are clearly more delicate in proportion to the extent of their monstrous modifications. If such a weakening is already present in the ordinary yellow fish to a slight extent, it could be determined only by an elaborate and long series of experiments. Evidently, the elimination of yellow fish works faster than any compensating behavior item leading to protective behavior of an adequate sort. It is difficult, however, to imagine what behavior would be useful in natural surroundings other than greater hiding proclivities. It must be borne in mind that most goldfish, even those raised in ponds, are protected to a considerable measure by man, and if there is any elimination taking place selectively, it is in the discarding of gray fish by the fish breeders. It would thus seem that there has been little, if any, chance of selection at a level which would permit the yellow fish to develop even a moderately useful compensatory behavior—perhaps just such a one as may be indicated by the experiments herewith reported. It would seem that under the conditions in which goldfish find themselves, either feral or cultured, the yellow ones are either early eliminated or protected in such a fashion as to preclude the selective survival of only those with a much modified behavior offsetting the disadvantage of their conspicuous coloration.

That like-colored fish and double pairs of differently colored fish show similar aggregating behavior patterns is understandable; but that the presence of a single odd-colored fish seriously disrupts what is essentially a rather regular behavior configuration is hard to explain other than to say that it evidently indicates that the fish are aware of differences in their fellows and that there is cohesion between differently colored fishes. The need for a postulate of "self-recognition," as given by Parr (1937), would hardly seem to be necessary. That this behavior is modified by isolation, on the other hand, is perhaps to be expected.

Parr (1927) oversimplifies the problem in speculating on whether schooling is an ontogenetic or phylogenetic matter. Clearly, elements of both an innate and a learned nature are present, and there is also both "habitus" and "heritage" involved. This is as should be expected, since schooling can be looked upon as the end-term of a long series of social attitudes ranging from solitary habits through every degree of aggregational activity, which is certainly derived from the totality—one might almost say the algebraic sum—of the factors in the social attitudes of a given species. Furthermore, probably in no two types is this sum, however similar appearing, the solution of the identical set of values.

CONSEQUENCES OF AGGREGATION

It is much easier to discuss the "how" of fish schools than the "why." As stated before, the evident thing that schooling behavior does is to keep large numbers of individuals in small compass. A critical examination of the possible benefits resulting from such a close association leads to the question of the utility to the species of this behavior.

If we resign from an attempt to ascribe a "useful" function to schooling or tentatively assume that there may be none, then the various items may be made to take on a uniform and reasonable appearance. As Parr (1927) points out in considerable detail, schooling is most conspicuous in fishes of a defenseless sort. Animals generally of this nature have but two alternatives for protection—flight or hiding. Benthonic fishes of defenseless types are given to hiding, apparently because there is present in their habitat something to hide behind, alongside of, or under. Surface fishes of a similar nature are given to similar behavior in so far as their cover is adequate. The behavior of goldfish in a well-planted lily pool will demonstrate this. When not close to some shelter, they seek protection in flight, but otherwise they hide under lily pads. Oceanic pelagic fishes show the same sort of behavior, except that there is little for them to hide behind except each other. Even so, floating trash, seaweed, boxes and barrels, large jellyfish, sharks, etc., at sea are generally attended by various species of small fish. If we attempt to defend the thesis that schooling is a reaction based on timidity, all the features herein discussed take on a rational appearance, and we need not inquire into the question of immediate utility. Almost certainly such fear reactions are valuable under certain conditions but under others, surely, neutral or harmful. Just what category any one school would fall under would be determined by features internal and external to the individuals at a particular time, and what might be disastrous on one day might be valuable on the next. Each presents a problem that would have to be studied, and no general statement which would cover more than the specific problem at hand could be made.

The present data on the reactions of yellow goldfish to a matching background, the fright schools of *Brachydanio*, and the behavior of mixed phenotypes all bear on these questions; but as yet no common denominator clearly appears.

A point that has not been explored, which was brought out in discussion by Dr. Beach, is the possibility of groups modifying the metabolism of an individual of a given species to some level necessary to its continued well-being. The data on the influence of companions on the oxygen consumption and locomotor activity of fishes is pertinent to this point. These findings are based on aggregating and not truly schooling species. It would be expected, a priori, that great-

er differentials would be found in truly schooling fishes, and it is conceivable that here they might be of such an extent as to have a rather immediate survival value. The difficulty that was found in rearing *Brachydanio* in isolation is suggestive in this connection. Shlaifer (1938) found that the color of associated goldfish had no effect on the respiratory behavior of individuals. All experienced aquarium keepers know that sometimes fish will refuse to feed to the point of starvation and that the addition of an actively feeding fish of the same or even another species will often be followed by feeding on the part of the others. Similar items have been found in isolated animals of other groups. If the mere finding of food is considered, it would seem that scattering would be more valuable. For example, a traveling school of mackerel engulfs such plankton as it may encounter. If, however, the school was more spread out, it would cover more area, and the school as a whole might be better fed, if there was any dearth of plankton. Since plankton tends to occur in streaks, this is the more pertinent. It would seem that schooling confers no direct advantage to the obtaining of nourishment. The effects of homotypically conditioned water, as studied by Allee (1935) and others, could hardly be expected to exert an influence in the open ocean.

The next great drive to be considered is that of sex, and here undoubtedly the quest for mates is reduced to a vanishingly small minimum, if both sexes are present in a school. However, actual counts of the sex composition of fish in schools are likely to be various. Although we need many more data on the subject to be able to say anything definitive, weakfish, for example, as taken in trap nets, run sometimes nearly all males and sometimes all females. How these are segregated we can only guess. At any rate, when taken on the spawning grounds, both sexes are present in approximately equal numbers. A hint may be had from the consideration of anadromous species, for here the males normally arrive first on the spawning beds, perhaps driven faster under the influence of male hormones. Nearly all fish and certainly all schooling fish about which we know anything make some sort of journey prior to spawning. This may be an extensive migration or simply a jaunt from the middle of a pond to its shore. Since the drive which causes them to move to their spawning site and the mechanism which enables them to find their way are evidently an individual matter, it would seem to make little difference whether they traveled in dense packs or as independent migrating individuals. Since no individual "knows the way" or acts as "leader" any better than another, the "advancing army" idea seems rather pointless.

Protection from enemies may be considered as a possibility, and this quickly reduces to a question in the mathematics of distribution. Given a roving pack of predators in search of food, do they find more or less in a given time if the latter is uniformly distributed or clumped? Without going into the theoretical mathematics of possible rates of collision under the two conditions, no very evident protection should be assured by this massing. It might be argued that the predators should be able to eat more if the prey was uniformly distributed, that is, catch one at a time at fairly well-spaced intervals instead of coming upon a large number and gorging. Any such effect would seem to be more than offset by the destructive manner in which such predators often feed. In their wake are usually left many unconsumed halves of fishes. It would seem that the mere presence of so many fish distracted attention

from the one already caught, causing it to be dropped while another flashing near by was similarly destroyed. Bluefish are notorious in this regard; see, for example, Breder (1925). A "confusion effect" was discussed by Welty (1934), following the introduction of an overabundance of *Daphnia* to goldfish.

If there are any other possible advantages to living in such large, closely integrated groups, they are not apparent at this time. The fact that all known schools are visual and are therefore interrupted by dark nights works against the assignment of any great continual and immediate value to them. Burkenroad (1943) made an interesting suggestion concerning the function of bioluminescence, but his effect would surely be a result of aggregation and not a cause thereof. Parr (1927), in discussing the mechanical features of the formation of milling schools—in which the fish simply go round and round but the group as a whole does not move forward—considered this a valueless performance. He did, however, consider the possibility of protection by schooling:

> If for instance the individuals of a much sought species like the sprat or herring would travel around separately, scarcely a single one of them would escape the enemies sufficiently long to be able to propagate, while the occurrence of a great number of specimens united in schools among scattered enemies may give a certain percentage a chance to survive and continue the existence of the species.

That he is not pressing this view very strongly is evident from his further statement:

> The essential problem, however, remains untouched by these theories. One might explain why the schooling performances are usually *not* observed among the benthonic fishes, the other why comparatively *few* pelagic species of a defenseless type are found to exist *without* the assumed protection of a schooling habit, but neither theory is able to shed any light at all upon the origin of the schooling behavior itself.

The further question of why schooling is so various in its aspects and in its presence or absence among different species would then seem referable to the physiological constitution of the animal in hand and thus becomes a basic query, somewhat similar to that of why some animals grow powerful teeth and jaws and are generally able to take care of themselves while others are feeble and depend on nonaggressive acts to escape destruction. There are, however, certain physiological leads that are worth considering. Eddy (1925) has already shown that aggregating impulses can be increased or decreased by chemical quantities in the environment, while the present studies could obtain such results only by methods destructive to the fishes. Reproduction, directly associated with changes in the chemical quantities in the blood (hormones), induces marked changes in the schooling reaction in some forms. In this connection the remarks of Parr (1931) are distinctly illuminating.

The ease with which free-swimming fish attain a "retinal vacuum" is not present in land animals. Presumably, the effects noted could be referred to a desire to avoid such a "blank" state. The positive phototropism exhibited by most species of a schooling nature may well be a reaction to the need of filling the visual field, which introduces another possible factor into the causes of school formation, which again may be with or without utility.

SUMMARY

1. Fright induced by a large variety of visual, mechanical, and chemical stimuli induces the formation of temporary schools in *Brachydanio rerio*, but any one stimulus soon loses its potency.

2. Attempts at training to aggregational behavior by a punishment technique led to fighting, instead of schooling, in *Brachydanio*.

3. Individuals of *Brachydanio* reared from the egg in isolation joined an aggregation at once, but individuals removed from a group to isolation and then later returned showed considerable hesitancy.

4. Moving targets of any shape were approached up to a certain size, beyond which they were retreated from, followed by a gathering at the point where last seen. Any such stimulus soon lost its potency for *Brachydanio*.

5. On being presented with equal areas of background, one yellow and the other gray, *Carassius* of either matching color avoided the yellow, although, in the case of the yellow fish, they became very conspicuous.

6. Groups of *Carassius* of like-colored phenotypes were more cohesive than groups in which one fish was of an odd color, on either matching or contrasting backgrounds, as measured by their aggregation pattern.

7. Isolation of *Carassius* for a period of 6 months modified the normal aggregating behavior pattern, and the fish appeared unusually sensitive to stimuli formerly ignored.

8. Phenotypically uniform groups of *Carassius* closely approached the distribution indicated by the reciprocal of binomial distribution, as contrasted with blind ones that approached the latter.

9. Mixed groups of blind and seeing *Carassius* approached the reciprocal of the mean of binomial distribution and its reciprocal.

10. Mixed groups of differing phenotypes of *Carassius* and groups of previously isolated *Carassius* and *Brachydanio* did not approach any evident simple mathematical expression.

11. The implications inherent in these results are discussed in reference to other items in fish aggregations and schools and speculations bearing thereon.

LITERATURE CITED

ALLEE, W. C. 1931. Animal aggregations. Chicago: University of Chicago Press. Pp. 431.

———. 1935. Relatively simple animal aggregations. Chap. xix. in CARL MURCHISON (ed.), A handbook of social psychology. Worcester: Clark University Press. Pp. 919+46.

———. 1938. The social life of animals. New York: W. W. Norton & Co. Pp. 293.

BOULENGER, E. G. 1929. Observations on the nocturnal behavior of certain inhabitants of the Society's Aquarium. Proc. Zoöl. Soc. London, Part I, pp. 359–62.

BOWEN, E. S. 1931. The role of the sense organs in aggregation of *Ameiurus melas*. Ecological Mono., **1**:1–35.

———. 1932. Further studies of the aggregating behavior of *Ameiurus melas*. Biol. Bull., **63**:252–70.

BREDER, C. M., JR. 1925. Growth of young bluefish. Bull. New York Zoöl. Soc., **28**:181–83.

———. 1929. Certain effects in the habits of schooling fishes, as based on the observation of *Jenkinsia*. Amer. Mus. Nov., No. 382, pp. 1–5.

———. 1932. On the habits and development of certain Atlantic Synentognathi. Papers Tortugas Lab., Carnegie Inst. Washington Pub. No. 435 **28**:1–35.

———. 1934. An experimental study of the reproductive habits and life history of the cichlid fish, *Aequidens latifrons* (Steindachner). Zoologica, **18**:1–42.

———. 1936*a*. The reproductive habits of the North American sunfishes (Family Centrarchidae). *Ibid.*, **21**:1–48.

———. 1936*b*. Fishes we don't exhibit—and why. Bull. New York Zoöl. Soc., **39**:149–53.

———. 1942. Social and respiratory behavior of large tarpon. Zoologica, **27**:1–4.

———. 1946. An analysis of the deceptive resemblances of fishes to plant parts. Bull. Bingham Oceanog. Coll. (in press).

BREDER, C. M., JR., and COATES, C. W. 1932. A preliminary study of population stability and sex ratio of *Lebistes*. Copeia, No. 3, pp. 147–55.

BREDER, C. M., JR., and NIGRELLI, R. F. 1935. The influence of temperature and other factors on the winter aggregations of the sunfish, *Lepomis auritus*, with critical remarks on the social behavior of fishes. Ecology, **16**:33–47.

———. 1938. The significance of differential loco-

motor activity as an index to the mass physiology of fishes. Zoologica, 23:1–29.

BREDER, C. M., JR., and RASQUIN, P. 1943. Chemical sensory reactions in the Mexican blind characins. Zoologica, 28:169–200.

BROWN, F. A., JR. 1936. Light intensity and melanophore response in the minnow, *Ericymba buccata* Cope. Biol. Bull., 70:8–15.

BROWN, F. A., JR., and THOMPSON, D. H. 1937. Melanin dispersion and choice of background in fishes, with especial reference to *Ericymba buccata*. Copeia, No. 3, pp. 172–81.

BURKENROAD, M. D. 1943. A possible function of bioluminescence. Jour. Marine Res., 5:161–64.

DEEGENER, P. 1918. Die Formen der Vergesellschaftung in Tierreiche: Eine systematisch-soziologischer Versuch. Leipsig. Pp. 420.

EDDY, M. W. 1925. Metabolic rate and bunching in catfish. Anat. Rec. 31:332.

ESCOBAR, R. A.; MINAHAN, R. P.; and SHAW, R. J. 1936. Motility factor in mass physiology: locomotor activity of fishes under conditions of isolation, homotypic grouping. Physiol. Zoöl., 9:66–78.

FRENCH, S. W. 1942. The effect of temperature on the retention of a maze habit in fish. Jour. Exper. Psychol., 31:79–87.

FRISCH, K. VON. 1938. Zur Psychologie des Fisch-Schwarmes. Naturwiss., 26:601–6.

———. 1941*a*. Die Bedeutung des Geruchsinnes im Leben der Fische. *Ibid.*, 29:321–33.

———. 1941*b*. Ueber eine Schreckstoff der Fischhaut und seine biologische Bedeutung. Zeitschr. f. vergl. physiol., 29:46–145.

GOZ, H. 1941. Ueber den Art- und Individualgeruch bei Fischen. Zeitschr. f. vergl. physiol., 29:1–45.

JONES, F. N. 1945. An alternative explanation of the effect of temperature upon retention in the goldfish. Jour. Exper. Psychol., 35:76–79.

KEYS, A. 1930. Influence of varying oxygen tension upon the rate of oxygen consumption in fishes. Scripps Inst. Oceanog. Bull., Tech. ser., 2:307–17.

LYON, E. P. 1905. On rheotropism. I. Rheotropism in fishes. Amer. Jour. Physiol., 12:149–61.

———. 1909*a*. Rheotropism in fishes. Biol. Bull., 8:238–39.

———. 1909*b*. On rheotropism. II. Rheotropism of fishes blind in one eye. Amer. Jour. Physiol., 24:244–51.

MAST, S. O. 1916. Changes in shade, color, and pattern in fishes and their bearing on the problems of adaptation and behavior, with especial reference to the flounders *Paralichthys* and *Ancylopsetta*. Bull. U.S. Bur. Fish. (1914), 34:175–238.

NEWMAN, E. 1876. Mr. Saville Kent's lecture, at the Society of Arts on "The aquarium: construction and management," Zoölogist, 11:4856.

NOBLE, G. K., and CURTIS, B. 1939. The social behavior of the jewel fish, *Hemichromis bimaculatus* Gill. Bull. Amer. Mus. Nat. Hist., 76:1–46.

PARR, A. E. 1927. A contribution to the theoretical analysis of the schooling behavior of fishes. Occas. Papers Bingham Oceanog. Coll., No. 1, pp. 1–32.

———. 1931. Sex dimorphism and schooling behavior among fishes. Amer. Nat., 65:173–80.

———. 1937. On self-recognition and social reaction in relation to biomechanics, with a note on terminology. Ecology, 18:321–23.

POPHAM, E. J. 1942. The variation in the colour of certain species of *Arctocorisa* (Hemiptera, Corixidae) and its significance. Proc. Zoöl. Soc. London, A, 111:135–72.

———. 1944. A study of the changes in an aquatic insect population using minnows as predators. *Ibid.*, 114:74–81.

RAND, A. L. 1941. Development and enemy recognition of the curve-billed thrasher, *Toxostoma curvirostre*. Bull. Amer. Mus. Nat. Hist., 78:213–42.

SCHNEIRLA, T. C. 1944. A unique case of circular milling in ants, considered in relation to trail following and the general problem of orientation. Amer. Mus. Nov., No. 1253, pp. 1–26.

SCHUETT, F. 1933. Studies in mass physiology: the effect of numbers upon the oxygen consumption of fishes. Ecology, 14:106–22.

———. 1934. Studies in mass physiology: the activity of goldfish under different conditions of aggregation. *Ibid.*, 15:258–62.

SCOTT, J. P. 1942. An experimental test of the theory that social behavior determines social organization. Science, 99:42–43.

———. 1944. Social behavior, range and territoriality in domestic mice. Proc. Indiana Acad. Sci., 53:188–95.

———. 1945. Social behavior, organization, and leadership in a small flock of domestic sheep. Comp. Psychol. Mono., 18:1–29.

SHAW, R. J.; ESCOBAR, R. A.; and BALDWIN, F. M. 1938. The influence of temperature and illumination on the locomotor activity of *Carassius auratus*. Ecology, 19:343–46.

SHELFORD, V. E., and ALLEE, W. C. 1914. Rapid modifications of the behavior of fishes by contact with modified water. Jour. Animal Behavior, 4:1–30.

SHLAIFER, A. 1938. Studies in mass physiology: effect of numbers upon the oxygen consumption and locomotor activity of *Carassius auratus*. Physiol. Zoöl., 11:408–24.

———. 1939. An analysis of the effect of numbers upon the oxygen consumption of *Carassius auratus*. *Ibid.*, 12:381–92.

———. 1941. Additional social and physiological aspects of respiratory behavior in small tarpon. Zoologica, 26:55–60.

———. 1942. The schooling behavior of mackerel: a preliminary experimental analysis. *Ibid.*, 27:75–80.

SHLAIFER, A., and BREDER, C. M., JR. 1940. Social and respiratory behavior of small tarpon. Zoologica, 25:493–512.

SPENCER, W. P. 1939. Diurnal activity rhythms in fresh-water fishes. Ohio Jour. Sci., 39:119–32.

SPOONER, G. M. 1931. Some observations on schooling in fish. Jour. Marine Biol. Assoc. United Kingdom, **17**:421–48.

SUMNER, F. B. 1934. Does "protective coloration" protect?—results of some experiments with fishes and birds. Proc. Nat. Acad. Sci., **20**: 559–64.

———. 1935*a*. Evidence for the protective value of changeable coloration in fishes. Amer. Nat., **69**: 245–66.

———. 1935*b*. Studies of protective color change. III. Experiments with fishes both as predators and prey. Proc. Nat. Acad. Sci., **21**:345–53.

———. 1944. Vision and guanine production in fishes. *Ibid.*, **30**:285–94.

———. 1945. The cause must have had eyes. Sci. Monthly, **60**:181–86.

SZYMANSKI, J. S. 1914. Eine Methode zur Untersuchung der Ruhe- und Aktivitätsperioden bei Tieren. Arch. f. d. ges. Physiol., **158**:359–65.

WALLS, G. 1942. The vertebrate eye. Bull. Cranbrook Inst. Sci., **19**:1–785.

WELTY, J. C. 1934. Experimental explorations into the group behavior of fishes: a study of the influences of the group on individual behavior. Physiol. Zoöl., **7**:85–128.

THE REACTIONS OF CULEX LARVAE AND PUPAE TO GRAVITY, LIGHT, AND MECHANICAL SHOCK

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ANYONE who has carefully observed mosquito larvae and pupae must have noted that they are very sensitive to a shadow, reacting by dropping down from the surface of the water. Both kinds of young are equally or even more sensitive to a mechanical disturbance, and they react in exactly the same way as to a shadow. Reactions of animals to mechanical disturbance and especially to reduction in light-intensity are well known to zoölogists. Mast (1911, p. 247) describes numerous observations made by himself and others on the effects of shadows on various animals, including mosquito larvae. He is of the opinion that such reactions are generally, if not always, adaptive and are useful either in protection or in the procurement of food. The reactions of mosquito larvae and pupae seem clearly to belong in the first category. On descending from the surface they hide among plants or debris, if such happen to be below them, and thus are doubtless often hidden from enemies. Incidentally, the reaction of the leech *Clepsine* (Whitman, 1898), which becomes restless and sways from side to side when subjected to a shadow, has been suggested as an example of a use of the response in obtaining food.

Despite the fact that the mosquito is of such great economic importance and that in many respects the behavior of itself and its young is so well known, little attention has been focused on those reactions of larvae and pupae to which we have just alluded. The promptness and vigor of the responses of the larvae and pupae to shadows, to mechanical shock, and, since the reactions involve up and down movements in the water, presumably also to gravity, would seem to make them desirable subjects for a study of the effects of these stimulating agents. This paper is concerned with such a study.

METHODS

The apparatus employed was simple. A museum jar of very clear glass, 18 cm. long, 15 cm. high, and 6 cm. wide, was supported, by means of a glass pane and wooden blocks, so that it was elevated about 6 inches above a shelf that ran along the side of a darkroom. Into this jar were put the larvae or pupae to be

experimented on. Two 150-watt lamps, mounted in stands with flexible necks, were placed one at the left end and the other above the jar, or one above and the other beneath it. These two lights were sometimes used singly, sometimes in combination. Occasionally, when both of the 150-watt lights were turned off, a weak red light of little or no stimulating efficiency was used for making observations.

Larvae and occasionally pupae of *Culex* comprised the experimental animals. In most instances 10, but sometimes 8, animals were put into the jar in a single test. Since the procedures differed considerably in the several experiments, further explanation will best be left until these experiments are described in detail.

EXPERIMENTAL RESULTS

Everyone is aware that both the larvae and pupae of mosquitoes must come to the surface to respire. In fact, in dishes in the laboratory, pupae remained almost constantly at the top; and larvae were usually there, though occasionally they dropped below. Observation of 270 larvae showed 225, or about 83 per cent, to be at the surface. Observation of 40 pupae showed all to be at the top, though rarely one does descend for a very brief period. These animals, of course, were undisturbed. As is well known and as we shall soon demonstrate, both larvae and pupae descend from the surface if subjected either to decrease in light-intensity or to mechanical shock. Under the influence of these stimuli there is apparently a reversion in the sense of orientation. But just what is involved in the reversion? It would appear that gravity must play a dominant role in determining the position of the animals, whether they are at the top or farther down; but apparently other forces—light, for instance—may also be involved. The experiments which will be described in the following pages were designed to ascertain how some of these forces act in conjunction with gravity in determining the position that young mosquitoes assume. The results of the first of the experiments are presented in Table 1.

In this experiment several groups, each containing 10 larvae, were put into a museum jar of the kind already described and were exposed to a 150-watt light, located at the left end of the jar, at the distances indicated. A piece of black paper, cut to the proper size and with a white line drawn through its center and perpendicular to its longitudinal axis, was inserted beneath the jar, thus dividing it into two equal compartments, the left one being nearest to the light, the right one farthest from it. During observations, counts were made of the animals in each compartment. These counts are recorded in Table 1 as decimal fractions, obtained by dividing the number of larvae in the half of the jar nearest to the light by the number in the half farthest from it. In this scheme, the figure 1 de-

TABLE 1

EFFECT OF UNILATERAL ILLUMINATION ON *Culex* LARVAE

Fractions obtained by dividing the number of larvae in the half of the jar next to the light by the number in the half farthest from it.

Group No.	Distance (in Cm.) from Light to Jar				
	120	90	60	30	10
1.....	1.00	0.61	0.54	0.50	0.33
2.....	0.94	1.07	0.94	0.50	0.40
3.....	0.62	0.76	0.46	0.63	0.15
4.....	0.82	0.54	0.36	0.88	0.71
5.....	1.08	0.67	0.56	0.43	0.06
6.....	0.67	1.77	0.67	0.92	0.14
7.....	0.47	0.43	0.39	0.73	0.56
8.....	0.39	0.28	0.28	0.19	0.14
Av...	0.75	0.77	0.53	0.60	0.31

notes indifference to light, less than 1 denotes negativity. As shown in the table, eight groups of larvae were used in the experiment, each group being subjected to five intensities. Five readings were made at each intensity. Intensity is indicated by giving the distance from light to jar. To alter its strength, the light was moved only a short distance; for example, it was moved from 120 cm. to 90 cm. and then to 60 cm., not directly from 120 cm. to 60 cm. This was to avoid making a greater change in intensity than was necessary—a precaution the necessity for which will be apparent later. Fifteen minutes were allowed for acclimatization at each intensity. A minute intervened between readings. Examination of Table 1 will show that all but a few of the groups were negative at all intensities and that every group was negative at the higher intensities. With the light at a distance of 120 cm. the averages from all eight groups produced a quotient of 0.75, indicating a small negative reaction. Little change is noted with the light removed to 90 cm., the quotient then amounting to 0.77. At 60 cm. it was 0.53; at 30 cm. it rose slightly to 0.60; and at 10 cm. it fell to 0.31. Thus, at this last intensity only about 3 out of 10 larvae were in the half of the jar next to the light. The group was very clearly negative. Nevertheless, while the animals do move away from the light because they are negative to it, certain modifying factors, not apparent from this experiment, are involved in the reaction. Since these will be made apparent by experiments soon to be introduced, further discussion will be deferred until later.

TABLE 2

EFFECT OF MECHANICAL SHOCK ON *Culex* PUPAE

Numbers indicate pupae that are below the surface. Observations every 15 seconds.

Group No.	Pupae below Surface									
1.....	0	0	0	0	Mechanical shock	8	5	1	0	0
2.....	0	0	0	0		10	6	1	0	0

As already mentioned, both larvae and pupae are extremely sensitive to mechanical shock. At the beginning of this work the stands with the lights were put on the same shelf as the jar containing the experimental animals. It was soon discovered, however, that with the stands in this position the vibration brought about by merely turning a switch sufficed to cause a violent reaction. So, to permit the turning on or off of the light without introducing a mechanical disturbance, the stands were transferred to a table, placed along the side of the shelf which supported the jar but not in contact with it.

The effect of mechanical shock is illustrated in Table 2, which presents the data from two tests on *Culex* pupae. In one test 8, in the other 10, pupae were put into the museum jar, with a light above it. Readings were taken every 15 seconds, counts being made of the number of animals that were below the surface film. In both cases, all were at the surface before stimulation. Mechanical disturbance, however, brought about by tapping the jar lightly with the fingers, caused all the animals in both groups to drop down immediately. But within 15 seconds some had returned to the top—3 in one case, 4 in the other. Fifteen seconds later all but 1 pupa in each group had risen to the surface, and within another 15 seconds all had risen. Thus, while the pupae react sharply to mechanical shock, they recover from its effects in less than a minute.

Mechanical shock has just as pronounced an influence on larvae as on pupae, and the preceding results are typical

for them also, except that it would be expected that 1 or 2 animals would be below the surface even when unstimulated and that, after stimulation, the return to the surface might not be quite so prompt.

TABLE 3

EFFECT OF DECREASE OF LIGHT-INTENSITY ON *Culex* LARVAE

(Numbers Indicate Larvae below the Surface)

	Group No.				Average
	1	2	3	4	
Observations every 30 seconds.....	0	1	1	2	1.00
	1	3	1	3	2.00
	2	0	1	4	1.75
	2	0	2	3	1.75
	(Light turned off for 2 seconds)				
Observations every 15 seconds.....	7	7	10	9	8.25
	4	6	8	8	6.50
	3	1	4	5	3.25
	1	0	2	3	1.50
	1	0	3	2	1.50
	1	0	2	2	1.25

A decrease in light-intensity also has a decided effect on the reactions of both larvae and pupae. This effect is well demonstrated in Tables 3 and 4. The procedures in the experiments recorded in these tables were identical, except that in one experiment larvae were made use of, in the other pupae. A 150-watt light was placed a few inches above the museum jar, which, in every instance, contained 10 experimental animals. At intervals of 30 seconds counts were made of the animals that were below the surface. The light was then turned off for about 2 seconds, turned on again, and more counts were made, this time at intervals of 15 seconds.

Table 3 shows tests on four groups of larvae; and, as may be seen, very similar results were procured in these tests. Before the light was turned off, the number of larvae below the surface averaged between 1 and 2. Immediately after it was turned off, most of them descended, and the average rose to 8.25. Quickly, however, they began to ascend; and in 15 seconds the average below the surface was 6.5, in 30 seconds it was 3.25, and in 45 seconds it was 1.5—essentially what it had been before the light was altered.

The experiment shown in Table 4, which consisted of tests on three groups of pupae, produced similar results. As might have been expected, no pupae were below the surface before turning off the light. Immediately after turning it off, however, an average of 7 had descended. As did the larvae, the pupae promptly began to ascend. In 15 seconds an average of 4.25 pupae remained down; in 30 seconds only 1.25 were below; and

TABLE 4

EFFECT OF DECREASE OF LIGHT-INTENSITY ON *Culex* PUPAE

(Numbers Indicate Pupae below the Surface)

	Group No.			Average
	1	2	3	
Observations every 30 seconds.....	0	0	0	0.00
	0	0	0	0.00
	0	0	0	0.00
	0	0	0	0.00
	(Light turned off for 2 seconds)			
Observations every 15 seconds.....	8	7	6	7.00
	4	5	4	4.33
	1	0	3	1.33
	0	0	0	0.00
	0	0	0	0.00

in 45 seconds none at all. Thus, both larvae and pupae respond quickly to a reduction in light-intensity, and both return to the surface in less than a minute after they have sunk down from it.

It is evident that both mechanical shock and decrease in light-intensity bring about a reversion in the response to gravity. Before stimulation the young mosquitoes are at the surface and presumably are negative to gravity; certainly they are not positive. As we have seen, stimulation by decrease in the intensity of light and by mechanical shock makes them temporarily very positive. However, in the experiments just described, a light was in position above the surface; and evidence has been presented showing that larvae are negative to a light—at least when it is at the side of them. This is not to imply that gravity may not have been involved in the foregoing reactions. It is easy to demonstrate that it is the deciding factor. Mechanical shock, for instance, brings about a descent from the surface, even in darkness. Nevertheless, light may have been a contributing factor. We shall next consider experiments designed to ascertain if light has any further effect on the position that young mosquitoes assume.

TABLE 5

EFFECT OF MECHANICAL SHOCK ON THE REACTION OF *Culex* LARVAE TO A LIGHT LOCATED AT ONE END OF THE JAR

Compartment on left nearest to light. Observations every 30 seconds.

Group No. 1				Group No. 2			
Compartment No.				Compartment No.			
1	2	3	4	1	2	3	4
5	0	2	1	3	0	2	3
4	0	1	3	3	0	1	4
3	1	1	3	2	1	0	5
3	1	1	3	2	1	2	3
3	1	1	3	2	1	0	5
(Mechanical shock)				(Mechanical shock)			
0	0	0	8	0	0	0	8
0	0	0	8	0	0	0	8
0	1	0	7	0	0	1	7
0	1	0	7	1	0	1	6
0	1	2	5	1	0	2	5
0	1	2	5	1	0	1	6
0	2	1	5	1	1	0	6

We have seen that, when undisturbed, larvae are moderately negative to a light placed at one end of the jar. We shall now consider an experiment which was an attempt to find out if mechanical shock has any effect on the reaction to a light when it is in this position. The results of the experiment are shown in Table 5 and were made on two groups, each composed of 8 larvae. These groups were put into a museum jar, underneath which was inserted a piece of black paper. Three white lines, drawn across the paper and viewed from above, divided the jar into four equal compartments; and with a light at the left end it was easy to observe its effects, simply by inspecting the numbers of animals in the compartments. A strong mechanical disturbance was created by picking up the jar, turning it around, and replacing it. Observations were made every minute. In the table the numbers in the left column show the animals in the compartment nearest to the light; those in the right column, the animals that were in the one farthest from it. Inspection of Table 5 will show that before mechanical stimulation there were, in both instances, about as many larvae at one end of the jar as at the other. After the disturbance, however, all animals in both groups promptly migrated to the compartment farthest from the light, actually crowding against the glass at that end. Here they tended to remain, though several min-

utes later a few had returned to the lighted end. Thus it is seen that mechanical shock makes larvae very strongly negative to light. But the fact that most of them remained away from the light for so long a period may not necessarily have been entirely due to a continued negative condition. After migrating, the larvae may have become indifferent and so remained where they happened to be simply through inertia. We shall return to this question after other data have been considered.

From the experimental results presented above, it is clear that light has a directive effect, just as does gravity, and that mechanical shock causes larvae to react definitely to light, just as it causes them to react to gravity. We have also seen that a decrease in light-intensity brings about a distinct response to gravity. One might well ask if it does the same thing to light, itself; that is: Will decrease in intensity cause light to have a directive effect? Observation has shown very clearly that it does. We have no data to present in the form of a table, but the reaction has been seen many times. It is best demonstrated by placing a light above the jar and another at the end. Turning off of the upper light causes the animals to drop down from the surface, as we have already seen, whereupon they swerve sharply from the light at the end. Indeed, turning the latter off and on will, by itself, bring about the same result, though then the effect is not so distinct, since not so many of the larvae descend from the surface.

In the two experiments next to be described the problem of the directive effect of light was attacked from a somewhat different standpoint. In these experiments two lights were employed—one above the jar, the other underneath it. In both experiments the lights were used separately, not simultaneously; in both, groups of 10 larvae or pupae were tested in the manner to be described; and in both, only animals that happened to be at the surface when the light was turned off or on were considered, since those below the surface did not react. Beyond this general account, a difference in the details of procedure makes it necessary to set forth each experiment separately.

In the first of these experiments, recorded in Table 6, groups of larvae or pupae were subjected either to an increase or to a decrease in intensity, with the light either above or below them. This repeated, in part, an experiment already described; but, since a considerable amount of new data has been added, the experiment will be presented in its entirety. As may be seen from the table, four groups of larvae and two groups of pupae were subjected to a decrease in intensity with the light above the jar. The decrease was brought about by turning off the light momentarily. Two or more observations were made on each group. In every group, either of larvae or of pupae, the majority of the animals that were at the surface responded. In four observations on group No. 1, for instance, 39 larvae were at the top when the light was turned off, 23 of them reacting. In the second group, 31 out of 38 larvae reacted; in the third group, 24 out of 36 reacted; and in the fourth group, 21 out of 25 reacted. Thus, out of a total of 138 larvae, 99, or 71.7 per cent, descended from the surface when the light was turned off. Two groups of pupae behaved similarly. Twenty-four out of 40, or 60 per cent, reacted to a decrease in intensity. The next tests, performed on three groups of larvae, were identical in procedure with those just described except for position of the light, which was

now beneath the jar. In the three groups a total of 86 larvae were at the surface when the light was turned off; 38 of them, or 44.2 per cent, reacted.

So far, we have noted only the effect of a decrease in light-intensity. As shown in Table 6, an increase may also be effective. In this case the 150-watt lights were turned off and the larvae exposed to a weak red light, which apparently had no stimulating efficiency and was thus equivalent to darkness. Increase in intensity was provided by turning on a 150-watt light, which was left on until counts could be made. As may be seen, three groups of larvae were tested with the light above the jar. Eleven animals, or 14.5 per cent, out of the total of 76, reacted. One group was tested with the light below the jar. None of the 23 animals that were at the surface responded.

TABLE 6

EFFECT OF DECREASE OR INCREASE OF LIGHT-INTENSITY ON YOUNG MOSQUITOES, WITH LIGHT EITHER ABOVE OR BENEATH JAR

Kind of Stimulus	Position of Light	Type of Animal	Group No.	No. of Animals at Top	No. of Animals Reacting	Percentage That Reacted
Decrease in intensity	Above	Larvae	1	39	23	
			2	38	31	
			3	36	24	
			4	25	21	
Total				138	99	71.7
Decrease in intensity	Above	Pupa	1	20	11	
			2	20	13	
Total				40	24	60.0
Decrease in intensity	Below	Larva	1	35	20	
			2	33	13	
			3	18	5	
Total				86	38	44.2
Increase in intensity	Above	Larva	1	26	2	
			2	34	7	
			3	16	2	
Total				76	11	14.5
Increase in intensity	Below	Larva	1	23	0	0.0

The foregoing results demonstrate again that young mosquitoes react sharply to a decrease in light-intensity. Moreover, it shows that they do so even if the light is below them. So far as the initial stimulus is concerned, the position of the light is probably of little consequence as, in all likelihood, when the light is below, the rays that are reflected down from the surface of the water cause the animals to react. If this is so, then the effective rays enter the eyes from the same direction irrespective of the position of the light, although they are weaker when the light is below, having then a somewhat longer distance to travel and there being fewer of them. It would seem, however, that the position of the light is significant in another respect. As already demonstrated, stimulation, either by mechanical disturbance or by decrease in luminous

intensity, makes young mosquitoes negative, so that they swerve strongly from a light that is at the side. When the light is above, this negative condition should be of efficacy in bringing the animals down from the surface; but if they are to react when it is below, the young mosquitoes must move directly toward the source of illumination, despite the fact that they are negative. Since they do react, it is obvious that the positive response to gravity is stronger than the negative response to light. Apparently this is not always true, since Jennings (1906, p. 150) states that, when other stimuli are opposed to gravity, it is the reaction to gravity that generally gives way.

The response of larvae to an increase in intensity is of interest. However, their failure to respond when the light was beneath the jar is probably of little significance. The reaction to an increase in intensity is not a very pronounced one, even with a strong light above. A low intensity, owing to the fact that to be effective the rays must be reflected downward from the surface, may well account for the lack of response when the light was below.

We have seen that, at times, larvae are negative to light. That at other times they are actually positive will appear from the results of the experiment recorded in Table 7. The procedure followed in this experiment has been described in part. Two lights were put into position—one above, the other underneath, the jar—and groups of 10 larvae were used as experimental animals. In contrast to the experiment just described, care was taken this time not to bring about a stimulation either by changing the intensity of the light or by mechanical disturbance, the purpose of the experiment being to compare the numbers of unstimulated animals habitually below the surface under certain contrasting conditions. Fifteen minutes were allowed for acclimatization to each condition. Counts were made at intervals of 1 minute. As may be seen from the table, the first comparison was between the number below the surface when there was a light above the jar and the number below when there was no light. In the latter condition, observations were made by means of the weak red light, mentioned earlier as equivalent to darkness.

TABLE 7

SHOWING AVERAGE NUMBERS OF LARVAE BELOW THE SURFACE UNDER CONTRASTING LIGHT-CONDITIONS

Ten larvae in each group. Numbers indicate those below surface.

Condition	Group No.					
	1	2	3	4	5	6
Darkness	2.37	1.2	....	....	.....	.....
Light at top	0.67	0.5	2.2	0.4	0.5	0.176
Light at bottom	.....	....	3.0	2.0	1.75	4.625
No. of observations under each condition	3	4	5	5	4	8

Two groups of larvae were used for the comparison. One group had an average of 2.37 larvae below the surface in darkness, as contrasted to 0.67 below in light. The other group had 1.2 larvae below in darkness, compared to 0.5 below in light. Apparently, unstimulated animals tend to be positive and so remain at the surface, as near to the light as possible. A comparison was also made between the number of larvae below the surface when the top light was on and the number below the surface when the bottom light was on. Four groups were used in this comparison. In the first group there was an average of 2.2 larvae below the sur-

face with the light above them, and an average of 3.0 below with the light beneath them. In the other groups the averages were 0.4, 0.5, and 0.18 with the top light on, and 2.0, 1.75, and 4.63, respectively, with the bottom light on. Again the light is seen to exert an attractive force, since there were decidedly more

TABLE 8

EFFECT OF AN INCREASE IN INTENSITY ON THE REACTION OF *Culex* LARVAE TO UNILATERAL ILLUMINATION

Observations every minute. Light at first at a distance of 110 cm. from the jar.

Group No. 1				Group No. 2			
Compartments				Compartments			
1	2	3	4	1	2	3	4
6	0	0	4	5	1	2	2
7	0	0	3	4	0	3	3
6	0	2	2	5	1	2	2
6	0	1	3	5	1	2	2
4	1	3	2	4	0	3	3
(Light moved from 110 cm. to 10 cm.)							
4	0	3	3	3	0	5	2
2	2	1	5	2	0	2	6
2	0	0	8	1	1	2	6
3	0	0	7	1	1	1	7
0	0	2	8	3	1	2	4
0	0	0	10	1	1	2	6
0	0	2	8	1	1	2	6
0	1	2	7	0	0	0	10

larvae below the surface when the light was underneath the jar.

One more experiment, presented in Table 8, will be described. In this experiment the piece of black paper employed in Experiment No. 4 was again inserted beneath the jar. A light was located at the left end, first at a distance of 110 cm., then at a distance of 10 cm. Counts, taken at intervals of a minute, established the distribution of the larvae throughout the length of the jar. Two groups of larvae were tested. With the light 110 cm. distant, it is seen that there were about as many animals at one end of the jar as at the other. When, however, it was moved quickly to within 10 cm. of the jar, the larvae very shortly began to migrate to the right, until finally, in both instances, all had moved as far away from the light as possible. In one group this condition was attained in 5 minutes; in the other group, in 8 minutes.

DISCUSSION

It is of interest to compare the reactions of young mosquitoes to those of *Drosophila*, whose extensive use in zoölogical laboratories has made its behavior as well known as that of the mosquito. As we have seen, mechanical shock renders mosquito larvae positive to gravity and negative to light. It just as clearly makes *Drosophila* negative to gravity and positive to light. This is easily demonstrated merely by picking up a bottle of the flies near a window and noting how the disturbance causes them to rush to the top of the bottle and to the side next to the light. Carpenter (1905), who studied the reactions of *Drosophila*, points out that two kinds of stimuli, or two phases of a single stimulus, are needed to bring about the foregoing responses. One kind is kinetic, that is, it induces movement; and the other kind is directive, that is, it determines the direction of movement. Obviously, in the example cited, mechanical shock is kinetic, and light and gravity are directive.

From what has gone before, it is evident that, as in *Drosophila*, both kinetic and directive stimuli are involved in the reactions of young mosquitoes. In one respect the reactions of the latter fail to parallel those of *Drosophila*. Carpenter maintains that the flies remain always positively phototropic and negatively

geotropic. He was unable to detect any sign of negative phototropism or of positive geotropism. As we have seen, there is a very definite reversion in the reactions of *Culex* larvae, both to light and to gravity.

In furthering the discussion on reversion, it might be well to note somewhat more in detail the behavior of larvae both before and after the response to a stimulus. Ordinarily, *Culex* larvae are found with the tip of the respiratory tube projecting through the surface film and with the body hanging down at an angle of about 45°. Occasionally an unstimulated larva—unstimulated, that is, by any external agent—will drop down from the surface, but it will soon return to the top. A strong external stimulus, such as we have seen exemplified, will cause most or all of the larvae to descend. In an unobstructed glass dish the larvae go down clear to the bottom and lie there, seemingly without effort. In ascending they frequently float upward, though the initiation of the upward movement may involve active swimming. The descent is always brought about by vigorous swimming. From this it would appear that the up and down movements in the water may be accompanied by changes in buoyancy. The larva is completely inactive when at the top, and apparently its specific gravity is then less than water, though it may be held up by surface tension. Certainly its specific gravity is less than water when it floats passively to the top. On the other hand, the fact that the larva lies quiescent on the bottom indicates that it has a specific gravity of not less than water. However, changes in buoyancy, if there be such, are perhaps of no special significance in the reactions with which we are concerned. However, one would like to know the condition of the quiescent animals with respect to light and gravity. Are they positive to the one and negative to the other when at the top, and the reverse of this when lying on the bottom? Our only criterion for ascertaining the sign of a reaction is direction of movement, and at these times there is no movement. Quite probably the larvae are indifferent when at the surface and, again, having reacted, while lying motionless beneath it. If this is true, they do not change immediately from positive to negative, or vice versa. However, the reversion is no less a reversion even though an indifferent stage intervenes between the two active phases.

Having seen that in the descent from the surface a kinetic stimulus may be involved, the question naturally arises as to whether such a stimulus is also involved in the rise to the surface. Certainly in those instances under observation no external agent has been concerned in the ascent; but this does not rule out the possibility of an internal stimulus, kinetic in its effect. One immediately thinks of lack of oxygen or accumulation of carbon dioxide as a kinetic agent, though, of course, there is no evidence for or against such a supposition. And for that matter, it is quite possible that an internal stimulus is usually concerned in the descent from the surface. The kinetic agents that we have studied—mechanical disturbance and change in light-intensity—are incidental in the life of the larva. Yet, as we have seen, the larva frequently drops down spontaneously. Gravity is always, light often, present as a directive stimulus. But if a kinetic stimulus is required at this time, it must be internal.

With regard to the stimuli that have been denoted "directive" and "kinetic," it is very clear that the latter is associated with time-rate of change and the

former with sustained action. From its very nature, mechanical shock involves rapid change and is kinetic in its effect. Gravity, on the other hand, must remain unaltered and can have only a directive effect. But light, which can change quickly or remain unchanged, should be, as it is, both kinetic and directive.[1]

A previous statement, that a light acting from below probably owes its kinetic effect to the rays that are reflected downward at the surface, may be recalled. Evidence for this in the present instance, it must be admitted, is circumstantial and is derived from the fact that larvae reacted much less strongly to a light that was below them than to one that was above. This could not have been due merely to a difference in intensity, since the light was as near to the animals in the one case as in the other. However, it seems quite possible that, because of the position of the larvae with respect to the surface, light may enter the eyes much more readily from above than from any other direction and that it is the light entering from above that exerts a stimulating effect. That the stimulating efficiency of a light may depend on the angle from which it enters the eye has been shown to be true in other organisms (Taliaferro, 1920; Dolley and Wierda, 1929; Mast, 1924).

As we have seen, larvae are negative to light when they drop down from the surface; that is, they are negative to light when they are positive to gravity. We have also seen that they can be positive to light. One might well ask whether they are positive to light when they are negative to gravity, that is, when they are rising to the surface. The positive reaction is by no means a strong one and is not clearly apparent by direct observation. It can be unmistakably demonstrated by means of only those methods that have been described, which permit a statistical study of data obtained by observation. Unfortunately, these data, while they do prove a positive reaction, do not indicate when it occurs. The results can be explained by assuming that, with a light above, for instance, either the larvae do not descend from the surface so frequently as they do when a light is below them or they do not remain down for so long a period. Direct observation seems to support the latter view. When there is a light at the side of them, rising larvae do appear to swerve toward it. If this is so, that is, if the rising larvae are positive, then with a light above them they should ascend more quickly than without a light, and with a light below them they should remain down longer. Obviously, when the light is above, its force as a directive stimulus is added to that of gravity; and when it is below, this force is opposed to that of gravity.

Finally, it may not be amiss to bring up again briefly and to correlate with other experimental evidence the results of the two experiments, one of which was presented in Table 1, the other in Table 7. It will be recalled that in both these experiments a light was placed at the left end of the jar; that, in both, observations were made to ascertain the distribution of the larvae with the light in that position; and that, in both, more than one intensity was employed, the lesser being that with the light at a distance of 110 cm. from the jar, the greater with it at a distance of 10 cm. In the first experiment, however, the larvae were thoroughly acclimated to each intensity,

[1] A kinetic effect of light has been observed in various organisms. Mast (1921) states, for example, that quiescent *Amaroucium* larvae are activated by a decrease in intensity, and Obreshkove (1921) has made the activation of frog tadpoles by an increase in intensity the basis for a quantitative study of the effects of light.

care being taken to avoid any quick changes; while in the other a rapid increase in intensity was involved, brought about by shifting the light from 110 cm. to 10 cm., with no time for acclimatization. It is not surprising, then, that differences in the distribution of the animals within the jar were discovered in the two experiments.

In the first experiment, the one illustrated in Table 1 and in which no quick change of intensity took place, it will be recalled that the larvae tended to collect toward the right end of the jar, away from the light, and that this tendency was strongest in the highest intensity. At no time, however, were all the animals over in the right half of the jar. Even at the highest intensity, about 3 out of 10 larvae were on the left side. It appears that when the larvae drop down, even though they do so spontaneously, they are negative and so swerve away from the light, but that, on rising, they are positive and move toward the light. The tendency toward aggregation in the part of the jar farthest from the light is apparently due to the fact that the positive reaction is considerably weaker than the negative one which precedes it. This positive reaction, while it does not completely neutralize the negative one, is, however, sufficient to prevent a complete migration to the side away from the light.

In the other experiment, not only were those forces involved which we have just described, but, in addition, a quick increase in intensity occurred. Though less potent than a decrease, we have seen that an increase in intensity can have a kinetic effect. That it had such an effect in this instance is attested to by the fact that all the larvae migrated to the right end of the jar, the end farthest from the light. That they did not migrate immediately, but only after an interval of several minutes, may have been due to the angle at which the light entered the jar, the larvae not being affected by it until they spontaneously descended from the surface.

SUMMARY

1. *Culex* larvae and pupae react to the turning-off of a light or to mechanical stimulation by dropping down from the surface of the water. Gravity is always involved in these reactions.

2. Two kinds of stimuli are joined to bring about the descent. One of these is kinetic and induces movement; the other is directive and determines the direction of movement.

3. A kinetic stimulus is associated with time-rate of change of the stimulating agent; a directive stimulus, with sustained action by it. Mechanical shock is kinetic in its effect; gravity is directive; and light, which can change quickly or exert sustained action, is both kinetic and directive.

4. Larvae react to a decrease in light-intensity whether the light is above or below them, but more strongly when it is above. Response to a light from below is probably due to rays reflected downward from the surface. This would, in effect, diminish the intensity and account for the lesser efficiency of the light in the lower position.

5. Larvae react, though not very strongly, to an increase in intensity if the light is above them. They fail to react if the light is below.

6. Larvae which are negative or indifferent to gravity when at the surface become positive under the influence of mechanical shock or change in light-intensity and descend. In a short time they revert to a negative condition and rise to the surface.

7. Apparently larvae are positive or indifferent to light when at the surface. Mechanical shock and change in intensity of illumination cause a reversion in the phototropic, as well as in the geotropic response; and under the influence of these stimuli the young mosquitoes become negative to light. Evidently they are photopositive when they are returning to the surface.

8. Though under the influence of a kinetic stimulus larvae become negative to light, they will descend even if a light is below them, showing that the geotropic response is stronger than the phototropic one.

9. The photonegative condition of the young mosquitoes is best demonstrated by placing a light at one end of the jar containing them, whereupon they swerve from it as they descend from the surface. The positive response can be demonstrated by comparing the number of animals habitually below the surface when a light is above it, with the number below the surface when there is no light, or by making a similar comparison with a light first above and then below.

LITERATURE CITED

CARPENTER, F. W. 1905. Reactions of the pomace fly, *Drosophila ampelophila* Loew, to light, gravity, and mechanical stimulation. Amer. Nat., **39**:157–71.

DOLLEY, W. J., JR., and WIERDA, J. L. 1929. Relative sensitivity to light in different parts of the compound eye in *Eristalis tenax*. Jour. Exper. Zoöl., **53**:129–39.

JENNINGS, H. S. 1906. Behavior of the lower organisms. New York: Columbia University Press. Pp. 366.

MAST, S. O. 1911. Light and the behavior of organisms. New York: John Wiley & Sons. Pp. 410.

———. 1921. Reactions to light in the larvae of the ascidians, *Amaroucium constellatum* and *Amaroucium pellucidum*, with special reference to photic orientation. Jour. Exper. Zoöl., **34**:149–87.

———. 1924. The process of photic orientation in the robber fly, *Proctacanthus philadelphicus*. Amer. Jour. Physiol., **68**:262–79.

OBRESHKOVE, VASIL. 1921. The photic reactions of tadpoles in relation to the Bunsen-Roscoe law. Jour. Exper. Zoöl., **34**:235–79.

TALIAFERRO, W. H. 1920. Reaction to light in *Planaria maculata* with special reference to the function and structure of the eyes. Jour. Exper. Zoöl., **31**:59–116.

WHITMAN, C. O. 1898. Animal behavior. Biol. lectures, Marine Biol. Lab., Wood's Hole, 1898, pp. 285–338.

EFFECT OF WEAK ALTERNATING-CURRENT SHOCKS UPON THE DIVISION OF STYLONYCHIA PUSTULATA (EHRENBERG)

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THE application of various types and strengths of weak electrical current to several genera of protozoans has resulted in a wide range of responses. Roesle (1903) described minutely the orientation of *Stylonychia mytilus* and other ciliates to single induction shocks. In several studies Kinosita (1936) has employed *Paramecium* in place of muscle for an exploration of electric polarization, the development of the latent excitation during stimulation, and

[1] The author wishes to acknowledge her debt of gratitude to the late Dr. Gary N. Calkins, of Columbia University, and to Dr. Benjamin P. Young, of Cornell University, for their kindly advice and sustained interest throughout this research. She wishes to thank, also, the directors of the Marine Biological Laboratory, Woods Hole, Massachusetts, and of St. Barnabas Hospital, Newark, New Jersey, for the use of their facilities.

other effects. Dierks (1926) used single induction shocks to reveal the nervous organelles of *Stentor coeruleus;* Richet (1927) investigated the lethal effect of a tetanizing current from the secondary coil of an inductorium upon fishes; Hutchings (1937) studied the effects of similar shocks upon division of *Stylonychia;* and Tittler (1938) explored regeneration and reorganization in *Uroleptus* after injury from shocks with a similar apparatus. With the exception of the preliminary studies by the author (1937), no one has yet investigated the effects of electricity on the division rate of a protozoan. Nor has anyone correlated the variations in division rate induced by electrical shock with the age of the culture from which the experimental animals were taken. It is now well known that the phases of growth in a protozoan culture (Phelps, 1935) are similar to those described for bacterial cultures by Buchanan and Fulmer (1928) and Henrici (1928).

Accordingly, cultures of *Stylonychia pustulata* (Ehrenberg) were grown in a standard manner, described below, and the life-cycle of such stock cultures was determined. Then, individuals from these cultures were exposed to 15-second shocks of 5 milliampere alternating current, and the effect on division rate was studied.

MATERIALS AND METHODS

Sufficient lettuce for all experiments was dried at one time in an electric oven. A 0.5 per cent medium was prepared from the lettuce, boiled, and ripened for 24 hours. Before autoclaving, one part of potassium monophosphate buffer solution (Giese, 1938) was added to every nine parts of medium. Healthy stylonychias were selected from a mass culture which had developed from a single isolated individual. After passing each animal through seven washes of sterile lettuce medium, following a modification of Parpart's (1928) technique, each one was placed in sterile lettuce medium containing a mixture of two or three kinds of bacteria. These bacteria had been obtained from the unwashed mass culture through bacteriological isolation technique. Those protozoans which underwent approximately three divisions in 24 hours at 26° C. became stock cultures. Although a few bacteria accompanied the washed stylonychias, the great preponderance of selectively inoculated food bacteria made the presence of other bacteria of little importance. When plated out every 3 weeks, *Aerobacter aerogenes*, *Proteus* (two species), and *Alcaligines* sp. outnumbered all others by a ratio approximating 60 to 1. For dishes containing experimental and control animals the incubator temperature was maintained at $25°.8 \pm 1°$ C.

New Columbia isolation culture dishes, whose concavity deepened to 5 mm. in the center, were used for exposing the protozoans to electric shocks. Each dish was washed with a mild soap, rinsed with distilled water, dried upon a lintless cloth, enclosed in a clean Petri dish, and sterilized in an electric oven at 180° C. for 1 hour. In use, each Petri dish became the individual moist-chamber of an isolation culture dish by distributing $1\frac{1}{2}$ cc. of distilled water around the outer rim of the lower half of the Petri dish. Also, to reduce dispersion of light, $1\frac{1}{2}$ cc. of distilled water were inserted between isolation dish and Petri dish.

In order to insure a relatively uniform amperage throughout the experiments, the quantity of medium which would permit 5 m.a. of alternating current to flow with the electrodes 9 mm. apart was determined for each isolation dish. A record of the correct amount was scratched

upon the glass culture dish with a carborundum point. Thereafter, exactly that quantity of medium was placed in an isolation dish, due allowance being made for the small quantity of medium transferred with the animal.

Electrodes 7 mm. in width by 15 mm. in depth of pure sheet platinum were spot-welded to No. 30 pure platinum wires. The wires were fused into a U-shaped bridge of solid glass, which permitted the electrodes to remain parallel and at the fixed distance of 9 mm. The outer ends of the platinum wires protruded beyond the glass bridge, which permitted the soldering of copper wire

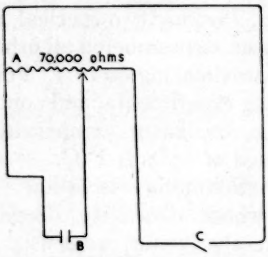

FIG. 1.—The electrical circuit

to the platinum wire. Copper wires made contact with the main circuit. Each electrode was slightly curved to conform with the shape of the cavity of the isolation dish. Between treatments of animals the electrodes were immersed in sterile lettuce medium. After using, electrodes were washed with alcohol, rinsed, and allowed to dry. In the 110 A.C. circuit, a 70,000-ohm radio potentiometer (Fig. 1, *A*) divided the voltage. By this means, current ranging from 340 microamperes to 22.5 m.a. could pass through the electrodes (Fig. 1, *B*). Precise regulation of amperage was accomplished through the attachment of a pointer and dial to the potentiometer. Gradations as fine as one-fifth of a milliampere were possible. A foot switch (Fig. 1, *C*), left both hands free for the manipulation of the culture dish and the electrode-bearing bridge. Five milliamperes of alternating current were applied for 15 seconds.

From a suitable stock culture an animal which showed constriction of the cytoplasm, indicating that fission would be completed in from 2 to 25 minutes, was removed by a sterile micropipette and placed in a sterile isolation dish enclosed in a sterile Petri-dish moist chamber. The time of separation (accurate to within 2 minutes) plus designation of size (small, medium, large) was noted. Two animals of similar size which had divided within 15 minutes of one another were designated as a "pair" and were given identical numbers. The percentage of a cubic centimeter (previously scratched upon the dish) of sterile lettuce medium needed to complete the requirement for uniform amperage described above was added, and all isolated animals were placed in an incubator at 25°.8 ± 1° C. The bacteria inoculated into the isolation dish along with the dividing organism multiplied, thereby providing food for the daughter stylonychias. From 4 to 6.5 hours later the pairs of dishes were carried to a laboratory for treatment, then replaced in the incubator. Since protozoans moving at the surface sometimes were destroyed by 15 seconds of 5 m.a. of current, a treatment was given only when both animals were moving upon the bottom of the dish and were, or could be maneuvered into, the center of it. The companion dish of the pair automatically became the control.

The results in both experimental and control dishes were read 24 hours after isolation, or 17.5–20.0 hours after the administration of the weak alternating current. The totals of the animals found in the experimental dish and of those in its companion control dish were recorded separately.

The stylonychias selected for a given day's work were not always taken from a stock culture in the same state, since a culture undergoes a sort of "life-cycle," which shows in the growth curve. In order to provide a correlation between the phase of growth of the culture and the stylonychia's response to electrical shock, a daily growth curve was determined for each stock culture. At approximately the same hour each day, the average percentage of dividing stylonychias in each stock culture was established. Every culture was moved slowly from left to right, and from the front backward, in such a manner that the entire Petri dish was covered systematically several times; and a total of 100 organisms was counted ten times. Occasionally, a count of 500 organisms had to be substituted for the customary average of 1,000 organisms per stock culture. Each stylonychia in which the macronuclei had fused together, forming a shining streak down the center, or which was in any later stage of fission, as shown by an obvious constriction, was counted as dividing. The average percentage of animals undergoing division in the ten counts was recorded as the percentage of division of the culture on that day. Cultures were used in experiments for 8 days. New stock cultures were established daily, often both night and morning, in sterile Petri dishes. In order to secure cultures which would pass through a vigorous growth cycle, new stock cultures were begun from animals which had shrunk to about one-third maximal size.

The animals used were always taken from the counted stock cultures. A curve based upon the average of the readings of each of 8 successive days has been constructed from an extensive series of readings of all stock cultures and is shown in Figure 2. On this graph the terminology used in connection with the usual protozoan growth curve has been associated with the corresponding region of a division percentage curve.

It may be noted that until after 24 hours following inception of the culture no animals could be found undergoing division. Thereafter the percentage of divisions rose rapidly, reached an average maximum of 15.3 divisions per 100 organisms 4.5 days after the starting of the stock culture, declined with equal rapidity, and became approximately zero in 8 days.

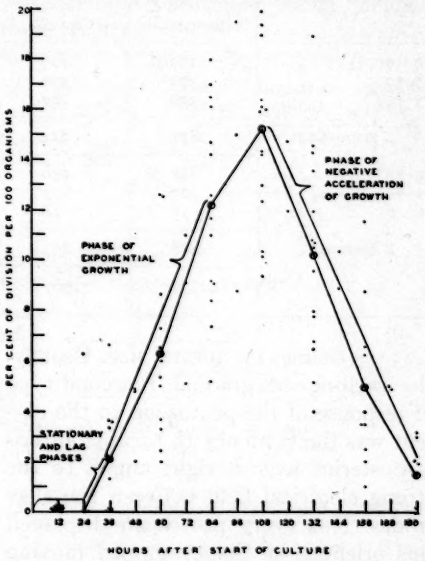
PER CENT OF DIVISION PER 100 ORGANISMS
PHASE OF NEGATIVE ACCELERATION OF GROWTH
PHASE OF EXPONENTIAL GROWTH
STATIONARY AND LAG PHASES
HOURS AFTER START OF CULTURE

FIG. 2.—Composite percentage-division curve. Average percentage in division per 1,000 organisms was computed daily. Twelve-hour readings are indicated but were not used in constructing the graph. Plotted points indicate an average of readings of all stocks.

RESULTS

I. VISIBLE EFFECTS OF THE ALTERNATING CURRENT

Through preliminary experimentation it became evident that the strongest electrical field in the dish was the 9-mm. gap between the parallel faces of the

electrodes. The most conspicuous visible effect of 5.0 m.a. of alternating current was the increase in volume of the protozoan. Each one swelled approximately

TABLE 1

NUMBER OF ANIMALS

Segments of Division Percentage Curve	No. of Animals	
	Experimentals	Controls
0– 6	193	178
7–12	372	354
13–19	306	282
Σ segment	871	814
19–13	246	258
12–7	207	217
6– 0	15	18
Σ segment	468	493
Σ	1,339	1,307

1.25–1.33 times its normal size. Usually, the swelling was gradual. A second type of response of the protozoan to the current was the tendency to turn its anterior-posterior axis at right angles to the strong electrical field between the electrodes. Not every protozoan displayed this orientation. Some stopped moving the instant that the circuit was completed. A third type of rerponse observed was spinning on the longitudinal axis, a reaction that might continue for several seconds.

II. EFFECTS OF ALTERNATING-CURRENT SHOCKS ON DIVISION OF *Stylonychia pustulata*

Selected stylonychias were given an electrical shock, allowed to multiply, and the number of divisions in the treated cultures compared to the controls. The results are shown in Table 1, which presents a simple summary in terms of number of animals produced in each segment of ascending and descending portions of the division curve.

It is interesting to note the similarity in column totals between the experimentals and controls. A chi-square test reveals, as expected, a lack of significance between 1,339 and 1,307 under the conditions of this test. Considering the total effect of the electrical treatment upon *S. pustulata*, one might conclude that it was nil. However, when one compares the segment total of 871 with 814, and 468 with 493, the differences appear to be significant. A chi-square test applied to the whole of Table 1 does not show conclusively that these differences could not have been due to chance. However, an analysis of variance performed upon data in Table 2 does prove that the difference in each region of the curve is significant.

In Table 2 the difference in actual numbers between experimental and control animals was established by subtracting controls from experimentals. The differences were grouped according to their location upon the ascending or the descending region of the division curve.

TABLE 2

DIFFERENCES IN NUMBERS OF ANIMALS

Differences in No. of Animals between Experimentals and Controls ($e-c$)	Regions of Division Curve	
	Ascending (0–19)	Descending (19–0)
+5	2	
+4	5	
+3	9	1
+2	15	7
+1	21	11
0	61	50
−1	19	17
−2	10	11
−3	6	4
−4	1	
Total	149	101
Mean	0.33	−0.23

A comparison of the means indicates an average gain of 0.33 in number of experimental animals over controls in the ascending curve and an average loss of 0.23 in number of experimental animals below the controls. An analysis of variance[2] was performed with the following results:

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares
Between means...	13.03	1	13.03
Within columns...	550.67	248	2.22

The value of F obtained is 5.85, which corresponds to a probability of approximately 0.02. Since only 2 times in 100 could such a difference occur by chance, the conclusion is that the difference in means is significant.

Table 3 presents the details of Table 2, displaying the differences between numbers of experimental and control animals in each segment of the ascending and descending regions of the curve. On analyzing the column means, it is interesting to note that in each case the columns for segment 7–12 and segment 12–7 have the means closest to zero.

In Table 4 the data on numbers of divisions have been treated in a manner comparable to that on numbers of animals in Table 2. Similarly, analysis of variance was performed which yielded these results:

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares
Between means...	2.51	1	2.51
Within means....	76.85	248	0.31

TABLE 3

DIFFERENCES IN NUMBERS OF DIVISIONS

Differences in No. of Animals between Experimentals and Controls ($e-c$)			Regions of Division Curve					
			Ascending			Descending		
			Segment 0–6	Segment 7–12	Segment 13–14	Segment 19–13	Segment 12–7	Segment 6–0
2	+5		.....	.....	2	.....	.....	.....
5	+4		.....	5	.....	.....	.....	.....
10	+3		2	2	5	.....	1	.....
22	+2		4	4	7	4	3	.....
32	+1		6	7	8	6	4	1
111	0		16	28	17	26	23	1
36	−1		3	8	8	7	8	2
21	−2		1	5	4	5	5	1
10	−3		.....	4	2	3	1	.....
1	−4		.....	.....	1	.....	.....	.....
Total 250		n	32	63	54	51	45	5
Mean.....			0.47	0.18	0.43	−0.24	−0.18	−0.60

Since an F of 8.1 was arrived at, corresponding to a probability of less than 0.01, there is less than 1 chance in 100 that such a result could have been obtained by chance. Therefore, the higher average number of divisions in the 0–19 ascending region of the curve is statistically different from the lower average number of divisions in the 19–0 descending region.

[2] R. A. Fisher, *Statistical Methods for Research Worhers.* (London: Oliver & Boyd, 1938).

Likewise, in Table 5 the analysis of number of divisions per segment of the curve is comparable to the treatment given in Table 3. It has been proved in Table 4 that there is a statistically significant difference between the halves of Table 5. The gain in number of divisions in the column for segment 13–19 appears to be the chief factor contributing to the difference, and the lower end of the descending segment seems to have the lower means. But there are not enough data to be sure that the columns on the descending segment are significantly different from one another.

TABLE 4

DIFFERENCES IN NUMBERS OF DIVISIONS

Differences in No. of Divisions between Experimentals and Controls ($e-c$)	Region of Division Curve		
	Ascending (0–19)	Descending (19–0)	Total
+2	1	.....	1
+1	32	7	39
0	98	75	173
−1	18	19	37
−2	.....	.....	.....
n	149	101	250
Mean	0.11	−0.12	−0.02

TABLE 5

DETAILED ANALYSIS OF DIVISION DIFFERENCES

Differences in No. of Divisions between Experimentals and Controls ($e-c$)		Regions of Division Curve					
		Ascending			Descending		
		Segment 0–6	Segment 7–12	Segment 13–19	Segment 19–3	Segment 12–7	Segment 6–0
1	+2	.....	.....	1	.....	.....	.....
39	+1	5	12	15	3	3	1
173	0	24	42	32	40	33	2
37	−1	3	9	6	8	9	2
Total 250	n	32	63	54	51	45	5
Mean		0.063	0.048	0.204	−0.098	−0.133	−0.20

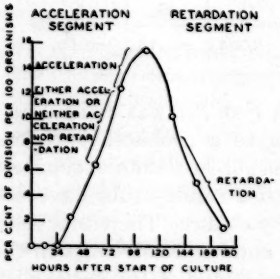

FIG. 3.—Characteristic response of *Stylonychias* to electricity in regions of division curve.

Finally, the results have been summarized in Figure 3 by associating with various segments of the division curve the characteristic average response of the experimental animals taken from that portion. The limits of each region are only tentatively suggested on the basis of the experimental evidence to date, but it is hoped that the problem may be much more adequately explored in the future.

DISCUSSION

McClendon (1927) concludes from a review of the literature that the plasma membrane is poorly permeable to ions but increases in permeability upon electrical stimulation. The most reasonable explanation of the swelling of *S. pustulata* following electrical treatment would seem to be that, because of the increased permeability of the membrane, fluid had entered the animal.

The essence of the findings reported here is that the effect which electrostimulation has upon division depends upon the phase of the "life-cycle" of the culture from which the experimental animal has been taken. If the protozoan is in the logarithmic phase, especially the late phase, there is a good chance of stimulation; if in the negative acceleration phase, it is likely to be retarded. It is quite possible that a correlation exists between the phase in the culture cycle and the rate of metabolic activity in *Stylonychia*. Thus, Hutchens (1941) in experiments upon *Chilomonas paramecium* showed that the oxygen consumption varied inversely with the age of the culture. In the same year Baker and Baumberger studied the respiratory rate of *Tetrahymena geleii*. As shown by a comparison of the curve of the rate of growth with that of the rate of consumption of oxygen, the time of high consumption of oxygen corresponds with the logistic (logarithmic) phase of growth, and the "stationary phase of the growth curve coincides in time with the slow change in the rate of consumption of oxygen" (Baker and Baumberger, 1941). In fact, their data show that, before the logarithmic growth phase is two-thirds complete, the rate of consumption of oxygen has diminished to one-half of its initial rate. What is true of the metabolism of *Chilomonas* and *Tetrahymena* is doubtless true of other protozoans, inasmuch as the growth curves of the protozoans studied to date are rather similar. Therefore, it is assumed that a reduced metabolism is characteristic of a culture of *S. pustulata*, which has a declining percentage of division. There remains one possible objection to this reasoning. Possibly a dividing cell always has a higher rate of respiration than a nondividing cell, even if it comes from a culture in which the average rate of oxygen consumption has declined. However, the author knows of no published results giving measurements upon single stylonychias or other dividing protozoans studied in microrespirometers.

The manner in which electrostimulation affects division is uncertain, but it may be that a moderately severe electrical shock creates a condition of injury characterized by a depressed metabolism, followed by a recovery process which proceeds at a high metabolic rate. In an animal having a high initial metabolic rate, the reconstructive processes, once they are set in motion, may proceed at a rate higher than normal. Then there is encountered an additive phenomenon. An abnormally high recovery metabolism could be superimposed upon an already elevated growth metabolism. Hence, it is not surprising to find that many stylonychias which are electrically shocked in the latter part of the exponential growth phase undergo more cell divisions than a comparable group of nonshocked control animals. In similar fashion, one may explain the retardation of divisions in stylonychias which are in the late stages of a declining division-rate phase. In an animal of slowed division rate, electrical shocks probably create a condition of injury. Since the metabolic rate is low, sufficient energy for the in-

stitution of a recovery metabolism is a long time in accumulating. Even though recovery from injury may proceed at a pace faster than the normal metabolism, perhaps so much time elapses before the quickened metabolism occurs that it causes the division rate of a group of electrically treated stylonychias from the later stages of the declining division phase to be lower than that of a normal control group.

The physicist Bethe (1940) has put forward the theory that "all rhythmic biologic processes are caused by repeated discharges of energy stored during the intervals between the discharges, a mechanism which can be compared best with a push-pull oscillator." He believes, further, "in biologic systems as well as in push-pull models, the discharge threshold depends on the characteristics of the system but the frequency depends on the charging velocity." If his theory is applied to the experiments described in this paper, the explanation of acceleration and retardation would be something like this: The energy for cell division is gradually stored during the major part of the interdivisional period. Upon the reaching of a certain threshold value, a mechanism of cell division is put into operation and fission takes place. If during the storing-up process the velocity at which energy is accumulated is increased, discharge of the system will occur more frequently. An increase in the velocity at which energy is stored probably is brought about through the intensification of metabolism by alternating current shocks. More frequent dissipations of the stored energy by cell divisions are to be expected. Since more frequent cell divisions actually took place in electrically treated stylonychias of young cultures, the experimental results are in accord with Bethe's theory.

SUMMARY

1. Individuals of the hypotrichous ciliate *Stylonychia pustulata* which have been treated with 15 seconds of 5 milliamperes of alternating current (*a*) increase temporarily in volume, (*b*) often spin under treatment, and (*c*) tend to orient between the electrodes so that the current passes across the short body axis rather than anterior-posteriorly.

2. There is no statistically significant difference between experimental and control groups as regards the total number of animals produced after such treatment.

3. Animals from the ascending segment of culture growth curve are more likely to be accelerated in number of animals and in number of divisions than to be retarded by treatment with weak alternating current. The reverse is true of *Stylonychias* selected for treatment from the descending segment of the curve.

LITERATURE CITED

BAKER, E. G. STANLEY, and BAUMBERGER, J. PERCY. 1941. The respiratory rate and the cytochrome content of a ciliate protozoan, *Tetrahymena geleii*. Jour. Cell. and Comp. Physiol., 17:285–97.

BETHE, ALBRECHT. 1940. Die biologischen Rhythmus-Phenomene als selbstandige bzw. erzwungene Kippvorgange betrachtet. Pflüger's Arch. f. d. ges. Physiol., 244:1–42.

BUCHANAN, R. E., and FULMER, E. I. 1928. Physiology and biochemistry of bacteria, 1:4–62. Baltimore: Williams & Wilkins Co.

DIERKS, KLAAS. 1926. Untersuchungen über die Morphologie und Physiologie des *Stentor coeruleus* mit besonderer Berücksichtigung seiner kontraktilen und konduktilen Elemente. Arch. f. Protistenk., 54:1–91.

GIESE, A. C. 1938. Race and conjugation of *Paramecium*. Physiol. Zoöl., 11:326–32.

HENRICI, A. T. 1928. Morphologic variation and the rate of growth of bacteria, pp. 17–45. Springfield, Ill.: and Baltimore: C. C. Thomas.

HUTCHENS, J. O. 1941. The effect of the age of the culture on the rate of oxygen consumption and

the respiratory quotient of *Chilomonas paramecium*. Jour. Cell. and Comp. Physiol., 17:321–32.

HUTCHINGS, L. M. 1937. Effect of electrical shocks upon the division rate of *Stylonychia pustulata* as measured by the interdivisional period. Biol. Bull., 73:386–87.

KINOSITA, HARUO. 1936. (*a*) Time-intensity relation of the cathodal galvanic effect in *Paramecium;* (*b*) Electric excitation and electric polarization in *Paramecium*. Jour. Fac. Sci., Tokyo Imp. Univ., 4:155–63.

———. 1938. Electrical stimulation of *Paramecium* with two successive subliminal current pulses. Jour. Cell. and Comp. Physiol., 12:103–17.

MCCLENDON, J. F. 1927. Colloid properties of the surface of the living cell; also Permeability and thickness of the plasma membrane as determined by electrical currents of high and low frequency. Protoplasma, 3:71–81; and Amer. Jour. Physiol., 82:525–32.

PARPART, A. K. 1928. The bacteriological sterilization of *Paramecium*. Biol. Bull., 55:113–20.

PHELPS, A. 1935. Growth of protozoa in pure culture. I. Effect upon the growth curve of the age of the inoculum and of the amount of the inoculum. Jour. Exper. Zoöl., 70: 109–28.

———. 1936. Growth of protozoa in pure culture. II. Effect upon the growth curve of different concentrations of nutrient materials. *Ibid.*, 72:479–96.

RICHET, CHARLES. 1927. Les Conditions de la mort par le tétanos électrique chez des poissons. Compt. rend. Acad. sci., 184:1100–1103.

ROESLE, E. A. 1903. Die Reaktion einiger Infusorien auf einzelne Induktionsschlage. Zeitschr. f. allg. Physiol., 2:139–68.

TITTLER, I. A. 1938. Regeneration and reorganization in *Uroleptus mobilis* following injury by induced electric currents. Biol. Bull., 75:533–41.

NEW BOOKS IN PHYSIOLOGICAL ZOÖLOGY

[This section comprises reviews of selected new books containing new and significant material in the field of physiological zoölogy. Books submitted for review should be sent directly to the review editor: Frank A. Brown, Jr., Department of Zoölogy, Northwestern University, Evanston, Illinois.]

PHYSICAL CHEMISTRY OF CELLS AND TISSUES,[1] BY RUDOLPH HÖBER, WITH THE COLLABORATION OF D. I. HITCHCOCK, J. B. BATEMAN, D. R. GODDARD, AND W. O. FENN

Reviewed by H. B. BULL
Northwestern University

THE Preface states: "The subject of this book is physiology; not 'physiology from above' but 'physiology from below'; not physiology originating essentially to fill human needs and help suffering individuals, but physiology as a branch of physical chemical sciences dealing with life as a physical, though exceedingly complex system, that may be subjected to scientific analysis like any other natural object."

The reviewer is very much in sympathy with the aims of this book. American science, in general, is far too concerned with "practical applications." Nature is a lady who must be wooed for her own worth before she will reveal herself. Certainly, there is a tremendous need for such a book as we have here and a still greater need for people with the viewpoint of the authors of this excellent text. It is a book which must be bought by all who wish to understand what is now known about many important phases of life-processes.

The first 91 pages were written by David I. Hitchcock and deal essentially with the physical chemistry of solutions. He discusses diffusion in solution, reaction velocity, thermodynamics, electromotive force, osmotic pressure, electrokinetics, and distribution coefficients. Hitchcock stays on broad and well-trodden highways. This section is clearly written, but parts of it are very scratchy and rather thin. For example, the reviewer doubts that a reader who is not already familiar with thermodynamics could profit very much by reading the chapter on this subject; too much territory is covered in the 12 pages. This brevity is carried to extremes in the discussion of dielectric constants, dipole moments, and the Deby-Hückel theory of strong electrolytes. Two pages are devoted to these subjects!

Section 2, which covers 120 pages, was written by J. B. Bateman. In this section Bateman deals with molecular forces, determination of sizes and shapes of large molecules, hydration, protein structure, X-ray diffraction analysis, the structure of crystals and fibers, and surface monolayers. There is much excellent material in this section. The part dealing with crystal structure is especially informative. Bateman covers many complex subjects, and the reviewer appreciates the

[1] Philadelphia: Blakiston Co., 1945. Pp. xiii+676+70 figs.+58 tables. $9.00.

great difficulty of presenting these topics simply; but it must be said that this section suffers from a lack of clarity.

The strictly biological part of the book begins with Höber's section (141 pp.), which deals with the penetration of cell membranes by organic nonelectrolytes, by electrolytes, by weak acids and bases, by dyestuffs, and by water; and, finally, he considers the chemistry and physics of plasma membranes. He brings to these subjects more than forty years of experience, and he has performed a great service to all of us in summarizing with completeness and detail the existing knowledge about cell membranes. Höber follows the Collander and Bärlund "lipoid-sieve theory" of cell membranes. He discusses active and passive uptake of substances and describes how they can be distinguished from each other. One cannot help having the feeling, after reading this section, that future progress in the study of cell penetration must involve a fuller consideration of the interior of cells. It appears that the penetration and action of narcotics is very obscure and would especially benefit by such expansion.

A section has been contributed by David R. Goddard (71 pp.) on the respiration of cells and tissues. Goddard's section is simply written, clear, and up to date. He deals with the rate of respiration as limited by the rate of diffusion of oxygen. The kinetics of activated reactions are discussed, and, after a short chapter on the nature of oxidation, he discusses respiratory enzymes. There follows a chapter on the relation between fermentation and respiration. Finally, there is a chapter on the utilization of liberated energy. This last is by necessity very incomplete, since our knowledge of this utilization is at present exceedingly imperfect.

W. O. Fenn has 75 pages on contractile tissues, with special reference to striated and smooth muscle. This is a well-balanced and critical review of experiments and theories of muscle contraction. It appears from this review that there still remains much to be known about this subject. Muscle physiology has passed through several phases, until now it has become a sophisticated science and one has to have a background in several branches of science to appreciate all the modern developments. One can, however, note considerable recent progress, and, as Fenn writes, "at last the contractile machinery has become more than a structure which is passively acted upon by lactic acid or some other metabolic product. It is as if steam had at last been admitted to the previously empty cylinders of the muscle machine."

Höber concludes the book with a section (110 pp.) on passive and active transfer in animal and plant tissues. In this section he deals with intestinal absorption, formation of urine, permeability of body surfaces of animals and plants, formation of digestive juices, and finally a discussion of some of the mechanisms which may be involved in active transfers. Here again, as in the section on cell membranes, there is a wealth of detail.

The book is well printed, and the reviewer noted only a few errors in it—and these were unimportant.

BIOENERGETICS AND GROWTH,[1] BY SAMUEL BRODY

Reviewed by J. H. BODINE
State University of Iowa

THIS book is an encyclopedic collection of growth data, treated and discussed in an exceptionally clear and comprehensive fashion. It is perhaps the only book in which data for all experimental animals, as well as for man, are completely and accurately assembled and compared. All phases of growth problems, basic as well as practical, are dealt with. In its approximately one thousand pages are some twenty-five chapters, each with well-chosen titles and summaries. Generous and complete references to pertinent data are included, as well as keyed footnotes. An excellent subject and author index is presented. Illustrations are numerous and well chosen. Graphs, with which the text is most liberally supplied, are well executed and clear. Many long tables containing data ordinarily difficult to obtain are found throughout the book. Their arrangements, style of type, etc., should make them of immeasurable value to those interested in varied problems of growth.

The subject matter of such comprehensive data is so treated that the book should be of great value to all interested in any phase of organic growth and especially to the experimentalist dealing with growth in any of its more dynamic phases. The text is clear and well written and the more technical and difficult questions of growth are handled competently. The chemical, physicochemical, mathematical, and physiological aspects of growth in all its various ramifications are included. The more practical aspects such as milk production, egg production, nutrition, and the like, are especially clearly discussed and illustrated.

The author is to be commended upon the production of a truly classic treatise on such a broad subject as growth.

[1] New York: Reinhold Pub. Corp., 1945. Pp. xii + 1023. $8.50.